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Health and Environmental Effects Profile for Styrene

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HEALTH AND ENVIRONMENTAL EFFECTS PROFILE FOR STYRENE

ENVIRONMENTAL CRITERIA AND ASSESSMENT OFFICE
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16. ABSTRACT

The Health and Environmental Effects Profile for styrene was prepared by the Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH for the Office of Solid Waste and Emergency Response to support listings of hazardous constituents of a wide range of waste streams under Section 3001 of the Resource Conservation and Recovery Act (RCRA) and to provide health-related limits for emergency actions under Section 101 of the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA). Both published literature and information obtained from Agency program office files were evaluated as they pertained to potential human health, aquatic life and environmental effects of hazardous waste constituents. Quantitative estimates have been presented provided sufficient data are available. Styrene has been evaluated as a carcinogen. The human carcinogen potency factor (q_1^*) for styrene is 2.47 $(mg/kg/day)^{-1}$ for oral exposure. Quantitative estimates have been presented provided sufficient data are available. Existing data are insufficient to determine an Acceptable Daily Intake (ADI) or a carcinogenic potency factor for styrene. The Reportable Quantity (RQ) value of 1, 10, 100, 1000 or 5000 pounds is used to determine the quantity of a hazardous substance for which notification is required in the event of a relase as specified by CERCLA based on chronic toxicity. Existing data are insufficient to determine an RQ value.

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PREFACE

Health and Environmental Effects Profiles (HEEPs) are prepared for the Office of Solid Waste and Emergency Response by the Office of Health and Environmental Assessment. The HEEPs are intended to support listings of hazardous constituents of a wide range of waste streams under Section 3001 of the Resource Conservation and Recovery Act (RCRA), as well as to provide health-related limits for emergency actions under Section 101 of the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA). Both published literature and information obtained from Agency program office files are evaluated as they pertain to potential human health, aquatic life and environmental effects of hazardous waste constituents. The literature searched and the dates of the searches are included in the section titled "Appendix: Literature Searched." The literature search material is current through November, 1984.

Quantitative estimates are presented provided sufficient data are available. For systemic toxicants, these include Acceptable Daily Intakes (ADIs) for chronic exposures. An ADI is defined as the amount of a chemical to which humans can be exposed on a daily basis over an extended period of time (usually a lifetime) without suffering a deleterious effect. In the case of suspected carcinogens, ADIs are not estimated in this document series. Instead, a carcinogenic potency factor of q_1^* (U.S. EPA, 1980) is provided. These potency estimates are derived for both oral and inhalation exposures where possible. In addition, unit risk estimates for air and drinking water are presented based on inhalation and oral data, respectively.

Reportable quantities (RQs) based on both chronic toxicity and carcinogenicity are derived. The RQ is used to determine the quantity of a hazardous substance for which notification is required in the event of a release as specified under CERCLA. These two RQs (chronic toxicity and carcinogenicity) represent two of six scores developed (the remaining four reflect ignitability, reactivity, aquatic toxicity and acute mammalian toxicity).

The first draft of this document was prepared by Syracuse Research Corporation under EPA Contract No. 68-03-3228. The document was subsequently revised after reviews by staff within the Office of Health and Environmental Assessment: Carcinogen Assessment Group, Reproductive Effects Assessment Group, Exposure Assessment Group, and the Environmental Criteria and Assessment Office in Cincinnati.

The HEEPs will become part of the EPA RCRA and CERCLA dockets.

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LIST OF ABBREVIATIONS

ADI	Acceptable daily intake
BCF	Bioconcentration factor
bw	Body weight
CHL	Chinese hamster lung
CHO	Chinese hamster ovary
CNS	Central nervous system
EEG	Electroencephalogram
i.p.	Intraperitoneal
LC_{50}	Concentration lethal to 50% of recipients
LD_{50}	Dose lethal to 50% of recipients
LOAEL	Lowest-observed-adverse-effect level
NOAEL	No-observed-adverse-effect level
ppb	Parts per billion
ppbC	Parts per billion carbon
ppm	Parts per million
RNA	Ribonucleic acid
SAP	Sensory action potential
SCE	Sister-chromatid exchange
SMR	Standardized mortality ratio
STEL	Short-term exposure limit
TLV	Threshold limit value
TWA	Time-weighted average

1. INTRODUCTION

1.1. STRUCTURE AND CAS NUMBER

Styrene is the common name for ethenylbenzene. It is also known as vinylbenzene, cinnamene and phenyethylene. The structure of styrene is given below:

Molecular formula: C_8H_8

Molecular weight: 104.16

The Chemical Abstract Services (CAS) registry number for this compound is 100-42-5.

1.2. PHYSICAL AND CHEMICAL PROPERTIES

Styrene is a colorless oily liquid with an aromatic odor (Hawley, 1977). It readily undergoes polymerization that could become violent when heated, exposed to light or in the presence of peroxide catalyst (Hawley, 1977). Some of the physical properties of styrene are given below (Verschueren, 1983, unless otherwise stated):

Melting point:	-30.6°C	
Boiling point:	145.2°C	
Density at 20°C:	0.9059 g/cm³	Lewis, et al., 1983
Solubility in water at 20°C:	300 mg/l	
Vapor pressure at 20°C:	5 mm Hg	
Log octanol/water partition coefficient:	2.95 3.16	Valvani et al., 1981 Banerjee et al., 1980

1.3. PRODUCTION DATA

Of the different methods that have been used or seriously considered for commercial production of styrene, the two most common methods are the reaction of ethylene and benzene in the presence of aluminum chloride to produce ethylbenzene that is catalytically dehydrogenated to styrene at ~630°C, and the oxidation of ethylbenzene to ethylbenzene hydroperoxide that reacts with propylene to give α-phenylethanol and propylene oxide. The alcohol is dehydrated to styrene (Lewis et al., 1983; Hawley, 1977).

The principal United States manufacturers of styrene, as listed by both the TSCA inventory (U.S. EPA, 1983) and USITC (1983), are given in Table 1-1. The data regarding production volume were obtained from the TSCA inventory (U.S. EPA, 1983), and the data regarding production capacity were obtained from SRI (1984). [The Cos-Mar plant at Carville, LA, was not listed in USITC (1983).] Two other plants owned by Koch Industries, Inc., Corpus Christi, TX, and U.S. Steel Corp., Houston, TX, with estimated annual capacities of 80 and 100 million pounds, respectively, remained idle due to market conditions (SRI, 1984). Although the total annual production in 1982 is shown to range from >3650-6100 million pounds, the actual United States production of styrene has been listed at >5942 million pounds by USITC (1983).

1.4. USE DATA

According to 1980 United States styrene consumption data reported by Lewis et al. (1983), the following percent of styrene was used in different industries: polystyrene, 63%; acrylonitrile-butadiene-styrene (ABS), 9%; styrene-butadiene elastomers (SBR), 9%; styrene-butadiene latices (SBL), 7%; unsaturated polyester resins (UPE), 6%; styrene copolymers containing >50% styrene by weight, 3%; polymers containing <50% styrene by weight, 2%; and styrene-acrylonitrile copolymer, 1%.

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TABLE 1-1

Principal United States Styrene Manufacturers in 1982*

Company	Range of Annual Production (million pounds)	Estimated Annual Capacity (million pounds)
America Hoechst, Baton Rouge, LA	NR	900
Amoco, Texas City, TX	100-500	790
Arco Polymers, Inc., Beaver Valley, PA	100-500	220
Houston, TX	100-500	1000
Borg Warner, Carville, LA	NR	NR
Cosden Oil and Chem. Co., Big Spring, TX	50-100	NR
Calumet City, IL	0.1-1.0	NR
Cos-Mar, Inc., Carville, LA	>1000	1300
Dow Chemical Co., Freeport, TX	>1000	1301
Midland, MI	100-500	324
El Paso Products Co., Odessa, TX	100-500	254
Gulf Oil Chem. Co., St. James, LA	100-500	600
Monsanto Co., Texas City, TX	>1000	1500
TOTAL	>3650-6100	8189

*Sources: USITC, 1983; SRI, 1984; U.S. EPA, 1983

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2. ENVIRONMENTAL FATE AND TRANSPORT

The fate of styrene in the atmosphere will be determined by its chemical and photochemical reactivity and the activity of atmospheric physical processes in the removal of this chemical. The rate constant for styrene reaction with singlet oxygen ($^1\Delta$) was studied by Datta and Rao (1979), and a rate constant for this reaction was 2.29×10^{-17} cm^3 molecule^{-1} sec^{-1} at 27°C. Assuming that the concentration of $O_2(^1\Delta)$ in air is 2×10^7 molecules cm^{-3} (Graedel, 1978), the half-life for this reaction at 27°C would be 48 years. Therefore, this reaction is not significant in determining the atmospheric fate of styrene.

The reactivity of styrene with ozone was studied by Hendry and Kenley (1979), and at 27°C the rate constant for this reaction was 1.71×10^{-16} cm^3 molecule^{-1} sec^{-1} and the half-life was 1.1 hours. The same reaction was also studied by Atkinson et al. (1982), and at 23°C the rate constant was determined to be 2.16×10^{-17} cm^3 molecule^{-1} sec^{-1}. Assuming that the atmospheric concentration of ozone is 1×10^{12} molecules cm^{-3} (Graedel, 1978), the half-life for this reaction would be 9 hours. Since the tropospheric average temperature is expected to be <23°C, the half-life of styrene in the troposphere due to this reaction may be >9 hours.

The kinetics of the reaction of styrene with OH• radicals was studied by Bignozzi et al. (1981), and the rate constant for this reaction at 25°C was determined to be 5.3×10^{-11} cm^3 molecule^{-1} sec^{-1}. If it is assumed that the concentration of OH• radicals in the troposphere is 10^{-6} molecule cm^{-3} (Atkinson et al., 1982), the half-life for this reaction would be ~3.6 hours.

The reactivity of styrene in the presence of NO_x and natural sunlight was studied by several investigators (Levy, 1973; Yanagihara et al., 1977; Korth, 1963). Yanagihara et al. (1977) determined that 90% of styrene present at an initial concentration of 2 ppm disappeared in 5 hours on irradiation with 700 $\mu W/cm^2$ of light at 345-355 nm wavelength and in the presence of 1 ppm NO_x. Similar smog chamber experiments by Korth (1963) with simulated sunlight (290-370 nm) and air-diluted auto exhaust providing styrene at simulated atmospheric concentrations showed a 55% disappearance of styrene in 2 hours.

The possibility of atmospheric removal of styrene through such physical processes as dry deposition and washout via rain or snow has not been comprehensively studied. Considering the relatively high chemical and photochemical reactivity of this compound in the atmosphere, however, it is unlikely that physical removal processes will play a significant role in determining the fate of this chemical.

The fate of styrene in natural aquatic media is likely to be determined by its ability to undergo chemical, photochemical and microbial reactions. Physical processes such as volatilization and sorption may also dictate the fate of this chemical in aquatic media. Information pertaining to the fate of this chemical due to reactions with available free radicals in water other than $RO\cdot_2$ are not available. The reaction of styrene with alkyl peroxy radicals was studied by Howard and Ingold (1968). On the basis of a rate constant value of 10 M^{-1} sec^{-1} (Howard and Ingold, 1968) and the concentration of peroxy radicals in natural water of 10^{-9} M, (Mill et al., 1981) the half-life for the reaction of styrene with peroxy radicals in aquatic media would be >800 days. Therefore, this reaction may not be a significant fate determining process for styrene in aquatic media.

Kinetic data regarding direct photochemical reactivity of styrene in aquatic media are not available in the literature. Styrene has a near continuous absorption from 220-300 nm, with an absorption coefficient of 15 ℓ mol^{-1} cm^{-1} in isooctane solution at 290.5 nm (Calvert and Pitts, 1966). Therefore, it is possible that photopolymerization may play a significant role in determining the fate of styrene in aquatic media. No quantitative prediction about the significance of this reaction can be made because of the lack of quantitative kinetic data.

The microbial degradation of styrene with isolated mixed microbes was studied by Sielicki et al. (1978) and Hou et al. (1983). Styrene was found to biodegrade with microbes isolated from landfill soil and propane-utilizing microbes isolated from lakes and soils. The possiblity of biodegradation of styrene in aquatic media was studied by Bridie et al. (1979a) and Price et al. (1974). It was shown by Bridie et al. (1979a) that 42% degradation of styrene (determined as theoretical biological oxygen demand) in water took place in 5 days, with unadapted filtered sewage seed as microbial inoculum. The microbial degradation was faster with adapted sewage seed, as 80% of degradation occurred in 5 days. Similar biodegradation potential of styrene in aqueous solution was reported by Price et al. (1974), who reported a 65% degradation of styrene in 5 days, with settled domestic wastewater as microbial inoculum. The degradation, however, was only 8% in synthetic saltwater under otherwise similar conditions. Although none of the above studies utilized ambient waters, they nonetheless indicate that styrene will be degraded relatively rapidly by microorganisms.

The reaction of chlorine with styrene present in water to form chlorohydrin may be significant during chlorination of drinking water. Kinetic data for this reaction were not located in the available literature.

Consequently, no quantitative value regarding the relative significance of this reaction can be assigned.

Styrene will volatilize from water relatively rapidly. Based on an EXAMS modeling (Burns et al., 1982) with input parameters as given by Lewis et al. (1983), the half-life for styrene removal through evaporation is estimated to be 3 days from a pond to ~13 days from an oligotrophic lake. Although no experimental data are available, based on the log octanol/water partition coefficient value of ~3, some styrene may be removed from aquatic media through sorption and subsequent sedimentation of particulate matter.

Zoeteman et al. (1980) estimated the overall half-life of styrene in river water to be 0.6 days, suggesting a fairly rapid disappearance >100 times faster than in groundwater, while their data in lake water indicated an intermediate rate of disappearance.

No experimental steady state BCF for styrene in aquatic organisms is available in the literature. The equation of Veith et al. (1979), log BCF = 0.85 log P-0.70, relating the BCF to the octanol/water partition coefficient (P), however, yields a BCF value of <100 for a log P value of 3.16 (Section 1.2.). Therefore, this compound is not likely to bioconcentrate significantly in aquatic organisms.

Of the limited studies available relating to the fate of styrene in soil, most pertain to the biodegradability of this chemical. Sielicki et al. (1978) reported that 95% degradation of styrene occurred with landfill soil in 16 weeks. The degradation of styrene was 87% with sandy loam soil during the same period of time. It was also shown by Sielicki et al. (1978) that the degradation was slower at higher doses (500 mg styrene in 100 g soil). The degradation of styrene in subsurface soil was found to be slow (Wilson et al., 1983). With two subsurface aquifers, only 2.3-12% degradation of styrene was noted per week.

The transport characteristics of styrene in aquifers was studied by Roberts et al. (1980), who reported that styrene was adsorbed relatively strongly by a sand aquifer. In cases where adsorption is responsible for the removal, the aquifer capacity ultimately is exhausted and breakthrough occurs. Styrene took 80 times longer to attain breakthrough in the aquifer than a nonadsorbing tracer such as chloride (Roberts et al., 1980). The investigation of Grossman (1970) of the contamination of groundwater by two drums of styrene buried in the soil (in Gales Ferry, CT) clearly demonstrates that styrene under certain conditions may leach through soil to groundwater. His data also show that styrene may persist in certain soils for at least 2 years.

Pertinent data regarding photochemical degradation on free radical reactions of styrene in soils were not located in the available literature. The volatilization of this compound from soil surface appears likely to be a significant loss mechanism; however, no information regarding the volatilization of this compound from soil was available in the literature searched.

3. EXPOSURE

3.1. AIR

The levels of styrene in the ambient air of the United States and other parts of the world are given in Table 3-1. It is difficult to assess the current level of styrene in the typical United States urban/suburban atmosphere away from stationary sources of styrene emissions either from Table 3-1 or from the additional data given by Brodzinsky and Singh (1982).

The levels of styrene near source areas have been determined by a number of investigators. Bozzelli et al. (1980) detected (not quantified) styrene in the atmosphere of a landfill in New Jersey. Ambient atmospheric styrene levels from the vicinity of six reinforced plastic processors located in Florida, Michigan and Ohio were measured by McKay et al. (1982). The concentrations of styrene adjacent to the plants ranged from 0.07-690 ppb. In communities farther away from these processors, styrene was detected at a concentration range of 0.4-5.6 ppb. In the Allegheny Mountain tunnel in Pennsylvania, the concentration of styrene due to vehicular traffic varied from 0.3-1.6 ppb (Hampton et al., 1983). The concentration of styrene in plumes over controlled forest fires was 2.5 ppbC (Westberg et al., 1981). About 3.5 miles downwind from the burn plume, the concentration was less than the detection limit (0.5 ppbC). Brodzinsky and Singh (1982) assessed the available data on the levels of styrene from different source dominated sites in the United States. Of these, data from 14 sites were assessed to have acceptable quality and the mean styrene concentration in these sites was 0.54 ppb.

The concentrations of styrene under certain occupational conditions were measured by Cocheo (1983). The concentrations of styrene in the vulcanization area of a shoe-sole factory, vulcanization area of a tire retreading

TABLE 3-1

Styrene Levels in Ambient Air Samples Around the World

Location	Date Sampled	Concentration (ppb)		Reference
		Range	Average	
Inglewood, CA	1965	8.0-15.0	11.5	Neligan et al., 1965
Long Beach, CA	1965	1.0-2.0	1.5	Neligan et al., 1965
Burbank, CA	1965	2.0-3.0	2.5	Neligan et al., 1965
Azusa, CA	1965	4.0-5.0	4.5	Neligan et al., 1965
Los Angeles, CA	1965	ND		Neligan et al., 1965
Kawasaki, Japan	NA	0.078-0.22		Nakayama et al., 1981
Leningrad, USSR	1976	detected		Ioffe et al., 1977
Six large cities, USSR	1977	detected		Ioffe et al., 1978
Delft, Netherlands	1975	ND to <0.7	<0.1	Bos et al., 1977
Eight cities, USA	NR	0.002-4.8		Pellizzari et al., 1979
Houston area, TX	1973-1974	1.94-4.44	3.38	Lonneman et al., 1979
Three urban sites, NJ	1981-1982	detected		Lioy and Daisey, 1983
Tuscaloosa, AL	1976	detected		Holzer et al., 1977
Talladega National Forest, AL	1976	detected		Holzer et al., 1977

factory, extrusion area of a tire retreading factory and extrusion area of an electrical cables insulation plant were measured at 21.2-117.5, 0.5-42.3, 0.2-4.7 ppb and none detected to 1.2 ppb, respectively.

The concentration range of styrene in United States ambient air is given as 1.5-5.0 ppb (0.4-1.2 μg/m³) by Graedel (1978). On the basis of this value and a value of 20 m³ of air breathed per day, the daily human intake of styrene through inhalation may range from 8-24 μg.

3.2. WATER

The levels of styrene in United States water samples monitored up to 1976 (Shackelford and Keith, 1976) are given in Table 3-2, which shows that styrene has been most frequently detected in industrial effluents. One of the most detailed cases of styrene contamination of potable water was documented by Grossman (1970). Sometime between 1959-1961, two drums of styrene were buried beneath 1-4 feet of landfill at Gales Ferry, CT, at the conclusion of the construction of a housing project. In 1962, six water wells in the area began delivering water with an obnoxious odor, which was due to the presence of 0.1-0.2 mg/ℓ styrene. In 1961-1962, all known contaminating material was removed from the ground and activated charcoal filters were installed on the water lines. The stryene concentration began to decline and was undetectable after 1964.

Dowty et al. (1975) reported the detection of styrene in drinking water passed through commercial charcoal-filtered units. Suffet et al. (1980) detected styrene in treated water collected from Torresdale Treatment Plant in Philadelphia, PA, in November, 1976.

3.3. FOOD AND OTHER INGESTED MATERIALS

Styrene has been monitored in food products and also in several non-nutritive materials that humans may ingest. Polystyrene food packaging is a

TABLE 3-2

Styrene Identified in Water[a,b]

1. Effluent from a latex plant (3/74), Louisville, KY
2. Effluent from a chemical plant (3/74), Louisville, KY
3. Effluent from a latex plant (10/75), Calvert City, KY
4. Effluent from a chemical plant (8/75), Calvert City, KY
5. River water (10/75), Hertfordshire SGI, England
6. Effluent from a chemical plant (8/74), Colliersville, TN
7. Finished drinking water (1970), LA
8. Effluent from a chemical plant (1970), Mississippi River, LA
9. Effluent from a textile plant (1970), Mississippi River, LA
10. Effluent from a chemical plant (C/73), Athens, GA
11. Finished drinking water (1/76), Cincinnati, OH
12. Finished drinking water, Evansville, IN
13. Effluent from a textile plant (2/75), Murray, KY
14. Effluent from a chemical plant (8/74), Memphis, TN
15. Finished drinking water (8/75), Grand Forks, ND
16. Finished drinking water, New York, NY
17. River water (7/75), Ames, IA
18. Finished drinking water (7/75), Ames, IA

[a]Source: Shackelford and Keith, 1976

[b]Monitoring dates, where available, are indicated in parentheses.

potential source of styrene monomer that may migrate into the food contained in the package. Container manufacturers have an impetus to keep monomer levels extremely low, not only because of the potential hazards involved in the ingestion of the monomer, but also because styrene imparts very undesirable odors to food at relatively low concentrations. For example, styrene can be detected in milk at 0.5 ppm and imparts a disagreeable odor and flavor to yogurt at 0.2 ppm (Santodonato et al., 1980). Milk stored in polystyrene containers for up to 8 days (presumably under refrigeration, although storage conditions were not specified) failed to show styrene in the milk. The lower limit of sensitivity of their analytical method was 0.05 ppm (Santodonato et al., 1980). By current standards, this is not especially sensitive. It was not indicated how much, if any, residual styrene monomer was actually present in the plastic containers used in the experiment.

In a detailed study of styrene in polystyrene packaging and food in contact with packaging, the levels of free styrene monomer in polystyrene food containers varied from 30-210 ppm (limit of detection was 1 ppm). The concentrations of styrene in a variety of dairy products in contact with polystyrene containers ranged from 20-80 ppb in yogurt (limit of detection was 0.91 ppb) to 140-240 ppb in sour cream (limit of detection was 13.4 ppb). The concentration of free styrene in food containers was well within FDA standards (10,000 ppm) (Santodonato et al., 1980).

In a recently conducted study (Eiceman and Carpen, 1982), styrene was detected at a concentration up to 270 ppm in polystyrene food containers. When water at 88-93°C was kept in contact with polystyrene cups, the maximum concentrations of styrene in hot water were 35 ppb after 15-20 minutes of contact time. Styrene was not detected in similar aqueous extracts with water at 20-25°C.

Styrene was detected in roasted filbert nuts, along with 227 other organic compounds (Santodonato et al., 1980). Styrene had not been previously reported in roasted filberts. No quantitative data were reported. The nut samples were not unusual in any way. The aim of the analyses was to identify molecules contributing to the characteristic filbert flavor rather than to seek contaminants.

Styrene has been found in four of seven samples of whiskey screened for organics. Two United States bourbon samples (3 and 4 years old) and one Canadian (unaged) sample of the seven did not contain styrene, whereas one other bourbon and two other Canadian whiskey samples (all unaged) and the condensate from a conventional beer still all contained styrene. Quantitative data were not reported (Santodonato et al., 1980).

Styrene was also detected in the volatile fraction of fried chicken flavor (Tang et al., 1983). The concentration of styrene in the fried chicken itself was not measured.

Finally, Pellizzari et al. (1982) monitored the styrene level in mother's milk from four urban areas at Bridgeville, PA, Bayonne, NJ, Jersey City, NJ, and Baton Rouge, LA. Styrene was detected in all the samples, although no quantitative data were provided.

Styrene is a component of cigarettes and cigarette smoke. It was found at a concentration of 18 μg/cigarette in a domestic filter blend. In addition, styrene was identified in the smoke. Styrene was also found in the air of an unventilated smoking chamber after a machine had smoked 30 American blend cigarettes. The concentration of the styrene was ~0.026 ppm (Sandtodonato et al., 1980).

4. PHARMACOKINETICS

4.1. ABSORPTION

Available data suggest that the absorption of styrene from the gastrointestinal tract is rapid and virtually complete. Peak blood levels were reached within minutes following oral intubation of rats with styrene in aqueous solution, while similar administration of styrene in vegetable oil resulted in peak blood levels ~100 minutes after dosing (Withey, 1976). Less than 10% of a 20 mg/kg dose of ^{14}C-styrene in oil solution was found in the gastrointestinal tract of rats 8 hours after gastric intubation (Plotnick and Weigel, 1979). Within 24 hours, <2% of the dose was found in the feces and ~90% had been excreted in the urine. Sauerhoff et al. (1976) found that fecal excretion accounted for only 4 and 1.5% of a 50 and 500 mg/kg dose, respectively, of ^{14}C-styrene administered in corn oil to rats.

Significant dermal absorption of styrene was demonstrated in humans (Dutkiewcz and Tyras, 1968). Absorption rates were 9-15 mg/cm^2/hour for liquid styrene and 40-180 µg/cm^2/hour for aqueous solutions of 66.5-269 mg/l applied to the forearms of male volunteers.

Absorption of inhaled styrene was reportedly rapid in both rats and humans. Withey (1976) detected measurable blood levels of styrene in rats within minutes after initiation of inhalation exposure to styrene concentrations ranging from 50-3000 ppm. Alveolar concentrations of styrene in humans reached plateaus ~1 minute after exposure began, indicative of rapid absorption (Astrand at al., 1974). The findings of a number of inhalation studies (Fiserova-Bergerova and Teisinger, 1965; Bardodej and Bardodejova, 1970; Fernandez and Caperos, 1977; Engstrom et al., 1978a,b; Teramoto and Horiguchi, 1979) indicate that pulmonary retention of styrene in humans is ~2/3 of the supplied concentration (mean uptakes ranged from 59-69%).

4.2. DISTRIBUTION

Plotnick and Weigel (1979) studied the distribution of styrene in rats following administration by gavage of 20 mg/kg ^{14}C-styrene. Peak tissue levels of radiolabel were reached within 2-4 hours of dosing. The kidney, liver and pancreas were the organs with the highest peak concentrations. Suprarenal fat also contained relatively high peak concentrations of radioactivity. Lower concentrations of radioactivity were found in the lungs, heart, spleen, adrenals, brain, testes, ovaries, muscle, bone marrow and skin. Twenty-four hours after dosing, all tissue concentrations measured were <1 µg/g. No styrene was detected after 48 hours. No radioactivity was detected by Sauerhoff et al. (1976) in tissues of rats 72 hours after oral administration of ^{14}C-styrene at dose levels of 50 or 500 mg/kg.

Teramoto and Horiguchi (1979, 1981) calculated organ/blood concentration ratios of styrene in rats following inhalation exposure. Adipose tissue had the largest ratio (by a power of 10) of all the tissues examined. Other investigators have also reported relatively high concentrations of styrene in adipose tissue of rats and mice following inhalation exposure (Withey and Collins, 1979; Carlsson, 1981; Bergman, 1979). In rats, the fat concentration of styrene increased 153-fold (from 34-5200 µg/g) with a 15-fold increase in exposure concentrations from 80-1200 ppm for $\leq$24 hours (Ramsey and Young, 1978; Young et al., 1979). These investigators found that the styrene concentration over time in fat was consistent with the pattern predicted for the peripheral compartment of a 2-compartment pharmacokinetic model. These results would be expected based on the high oil-blood partition coefficient of styrene, which was estimated as 130 (Van Rees, 1974) or 105 (Sato and Nakajima, 1979).

In addition to adipose tissue, relatively high concentrations of styrene and its metabolites have been detected in liver tissue followed by brain, kidney, spleen and muscle tissue of rats (Teramoto and Horiguchi, 1979, 1981) in kidneys, liver and the ischiadic nerve of rats (Carlsson, 1981) and in nervous tissues of mice (Bergman, 1979). Withey and Collins (1979) found that the relative concentrations of styrene in the liver and brain of rats increased as the inhalation exposure level of 50 ppm for 5 hours was increased to 2000 ppm for 5 hours. Transplacental transfer of styrene in laboratory animals has been confirmed by Grice et al. (1981). Mean tissue levels of ~38-65 µg styrene/g bw were found in the offspring of pregnant rats exposed to 2000 ppm styrene for 5 hours on day 17 of gestation.

The relatively rapid elimination of styrene from all tissues, which was observed after oral exposure (Plotnick and Weigel, 1979), is supported by results using other routes of exposure (Withey and Collins, 1977; Teramoto and Horiguchi 1979, 1981; Carlsson, 1981; Pantarotto et al., 1980; Bergman, 1979). Teramoto and Horiguchi (1979, 1981) estimated the biological half-life of styrene in adipose tissue as 6.3 hours following a 4-hour inhalation exposure of rats to 500-1000 ppm styrene. Half-lives ranged from 2-2.5 hours for all the other tissues examined. Five daily 4-hour exposures to 700 ppm styrene did not result in significant accumulation of styrene (Teramoto and Horiguchi, 1979, 1981).

Human tissue concentration data are limited to biopsies of subcutaneous adipose tissue following inhalation exposure. Adipose tissue samples from volunteers exposed to 50 ppm styrene during four 30-minute periods of rest and exercise contained mean concentrations of ~36 mg styrene/kg during the 24 hours following exposure (Engstrom et al., 1978a). A mean daily uptake of styrene in adipose tissue was estimated as 193-558 mg styrene in three

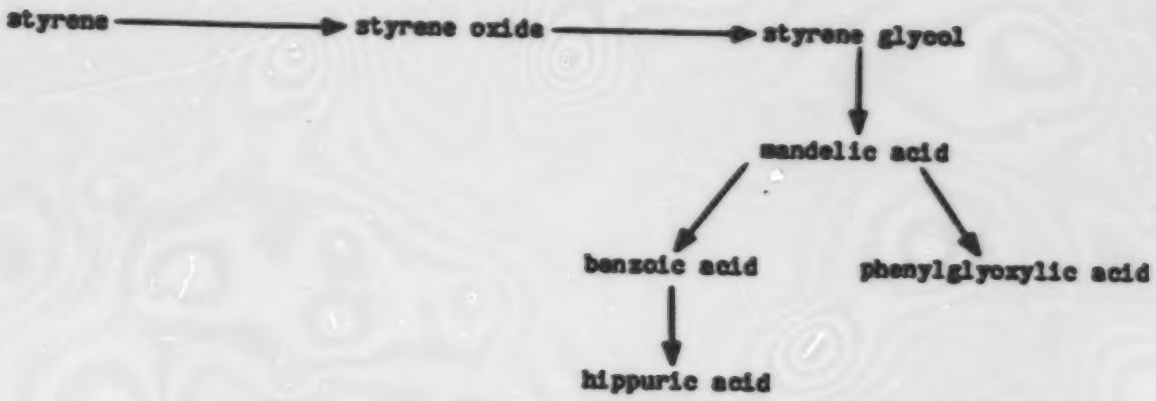
workers exposed to TWA concentrations of 7.6-20 ppm styrene (Engstrom et al., 1978b). Dowty et al. (1976) measured concentrations of transplacental styrene which were higher than the concentrations in maternal blood.

Engstrom et al. (1978a) estimated half-lives of 2-4 days for styrene in adipose tissue of humans exposed by inhalation to 50 ppm styrene for 2 hours. Predictions based on a pharmacokinetic model using parameters estimated from a human inhalation study revealed no tendency for long-term accumulation of styrene (Ramsey and Young, 1978, 1980; Ramsey et al., 1980). The predictions indicated that the maximum concentrations of styrene in both blood and fat of humans are reached after a few days of exposure to 80 ppm styrene x 8 hours.

4.3. METABOLISM

The metabolic fate of styrene in mammals has been well studied. Ohtsuji and Ikeda (1971) found that i.p. injection of styrene in rats resulted in urinary excretion of mandelic, phenylglyoxylic and hippuric acids and glucuronide. Injection of styrene-7,8-oxide or styrene glycol resulted in the same urinary metabolites. Injection of phenylglyoxylic acid resulted in excretion of only phenylglyoxylic acid, while mandelic acid injection yielded all three acids. The metabolism of styrene was stimulated by pretreatment with phenobarbital (a microsomal cytochrome P-450 inducer) and was inhibited by the microsomal oxidation inhibitor, SKF 525A. Phenobarbital pretreatment did not affect styrene oxide or styrene glycol metabolism. The

following metabolic pathway was proposed (Ohtsuji and Ikeda, 1971) based on the results summarized above:



Styrene glycol may alternatively be excreted as a glucuronide conjugate (Ohtsuji and Ikeda, 1971).

The results of Leibman and Ortiz (1970) indicated that the enzyme catalyzing the conversion of styrene to its oxide is the microsomal NADPH-cytochrome P-450-dependent monooxygenase. Styrene oxide, in turn, may be hydrated to styrene glycol by a microsomal epoxide hydrase (Leibman and Ortiz, 1968). An alternate metabolic pathway that was suggested by the presence of hydroxyphenyl mercapturic acids as urinary metabolites (James and White, 1967) involves the conjugation of styrene oxide with reduced glutathione (GSH). This conjugation was catalyzed <u>in vitro</u> by cytosolic glutathione-S-transferase (Boyland and Williams, 1965; Fjellstedt et al., 1973).

The major pathways in styrene metabolism are the two outlined above. Metabolism of styrene to styrene-3,4-oxide, which is subsequently converted to 4-vinyl phenol, has been proposed as a minor pathway based on studies by Pantarotto et al. (1978) and Watabe et al. (1982). Low levels of 4-vinyl

phenol were detected in the urine of rats following oral administration of styrene (Bakke and Scheline, 1970). Detection of additional urinary metabolites has led to the proposal of several other minor pathways (Nakatsu et al., 1980; Horning et al., 1981; Battistini et al., 1979; Delbressine et al., 1980).

In humans, the primary urinary metabolites of styrene have been identified as mandelic and phenylglyoxylic acid (Bardodej and Bardodejova, 1970; Astrand et al., 1974; Guillemin and Bauer, 1978, 1979). Pfaffli et al. (1981) identified 4-vinyl phenol in the urine of plastic workers exposed to a TWA of 130 ppm styrene. The mean 4-vinyl phenol to mandelic acid excretion ratio was ~0.3%, which suggests that the pathway to 4-vinyl phenol is minor.

4.4. EXCRETION

Over a 72-hour collection period, 95% of a 50 mg/kg oral dose of ^{14}C-styrene administered to rats was excreted in the urine. 1% was expired and 4% was recovered in the feces (Sauerhoff et al., 1976). Urinary excretion accounted for 90% of a 500 mg/kg dose, while 9% was expired and only 1.5% was eliminated in the feces. Four of the seven urinary metabolites of styrene detected were mandelic, phenylglyoxylic, hippuric and benzoic acids. Plotnick and Weigel (1979) found that 90% of a 20 mg/kg oral dose of ^{14}C-styrene was excreted in the urine within 24 hours. Urinary excretion also accounted for ~90% of the radioactivity following inhalation exposure to ^{14}C-styrene, while 6% was expired and 2.3% was recovered in the feces (Sauerhoff and Braun, 1976). Urinary excretion of mandelic and phenylglyoxylic acid accounted for 90-97% of the retained dose after inhalation exposure of humans (Guillemin and Bauer, 1978, 1979; Ramsey and Young, 1978, 1980; Ramsey et al., 1980).

Rapid and biphasic log-linear elimination of styrene in expired air, urine and from the blood has been observed in animals at low doses (Sauerhoff et al., 1976; Withey, 1976; Sauerhoff and Braun, 1976; Bergman, 1979; Ramsey and Young, 1978, 1980; Young et al., 1979; Withey and Collins, 1977, 1979). At higher doses the shape of the blood and urinary elimination curves indicates saturation of metabolism or excretion (Sauerhoff et al., 1976; Ramsey and Young, 1978, 1980; Young et al., 1979; Teramoto and Horiguchi, 1979, 1981). For example, following an oral dose of 50 mg/kg, urinary excretion in rats was biphasic, with half-times of 1.3 and 8 hours for the alpha and beta phases, respectively (Sauerhoff et al., 1976). At a higher dose of 500 mg/kg, styrene excretion peaked between 12 and 24 hours after dosing, then declined linearly with a half-time of 7 hours. Biphasic elimination has been observed in humans with plasma alpha and beta half-times estimated as 0.6 and 13 hours, respectively, following inhalation exposure to 80 ppm styrene x 6 hours (Ramsey and Young, 1978, 1980; Ramsey et al., 1980). Guillemin and Bauer (1978, 1979) measured urinary mandelic acid half-times of ~4 and 25 hours for the alpha and beta phases, respectively, in humans exposed to 50-200 ppm styrene for 4-8 hours.

5. EFFECTS

5.1. CARCINOGENICITY

The information available regarding the potential carcinogenicity of styrene is limited and equivocal. Several of the long-term animal bioassays were characterized by excessive mortality among treated animals and inconsistent observations of elevated tumor formation.

Ponomarkov and Tomatis (1978) administered styrene (99% pure) in olive oil by gavage at doses of 1350, 300 or 1350 mg/kg to 29 female O_{20} mice, 15 female C57BL mice and 21 female BD IV rats, respectively, on day 17 of gestation. Nine O_{20} mice, 5 C57BL mice and 10 BD IV rats served as vehicle controls. After weaning, the offspring of the C57BL mice and BD IV rats received weekly doses for life of styrene at levels of 300 or 500 mg/kg. Weekly dosing of the offspring of the O_{20} mice with 1350 mg/kg was discontinued at 16 weeks because of overt toxicity.

There were no statistically significant differences in survival or tumor incidences in the 27 male and 27 female styrene-treated offspring of the C57BL mice or the 73 male and 71 female offspring of the BD IV rats compared with their respective controls. A few tumors observed in treated rats were rarely observed in controls (3 stomach tumors, 1 liver tumor and 3 neuromas). The average age at death was much lower in treated offspring of the O_{20} mice than in controls (32 and 49 weeks for treated males and females, compared with 88 and 85 weeks for male and female controls). The incidence of lung adenomas and carcinomas in treated O_{20} mice offspring was significantly increased over vehicle controls. Based on survivors at the time of the first observed tumor, the incidences were 20/23 and 32/32 in treated males and females compared with 8/19 and 14/21 in male and female controls.

In a National Cancer Institute bioassay, styrene was administered by gavage in corn oil to Fischer 344 rats and B6C3F1 mice 5 days/week for 78 weeks, followed by a 25-week observation period (NTP, 1979). Groups of rats (50/sex) received doses of 1000 or 2000 mg/kg/day, while mice (50/sex/group) were given 150 or 300 mg/kg/day. A dose of 500 mg/kg/day was administered to an additional group of rats for 103 weeks and 20 animals of each sex served as vehicle controls. Early mortality in rats dosed with 2000 mg/kg precluded analyses of tumor incidence. There were no significant increases in any tumor type in male or female rats at the other dose levels or in female mice. Male mice at the 300 mg/kg dose level exhibited an increased incidence of alveolar/bronchiolar carcinomas and adenomas. This incidence (9/43 or 20.9%) was significantly higher than the incidence in the vehicle controls (0/20), but was elevated less markedly in comparison to the historical control incidence of 12%.

Maltoni et al. (1982) investigated the potential of styrene to induce brain tumors by treating Sprague-Dawley rats (40/sex/group) by gavage with styrene in olive oil. Styrene was administered 4-5 days/week for 52 weeks at dose levels of 0, 50 or 250 mg/kg. Examination after spontaneous death did not reveal any significantly elevated incidences of brain tumors in treated rats compared with vehicle or historical controls. Maltoni et al. (1982) also found no evidence to suggest that styrene induces brain tumors in Sprague-Dawley rats following inhalation exposure. Rats were exposed to concentrations up to 300 ppm 4 hours/day, 5 days/week for 52 weeks.

In a chronic inhalation bioassay, Jersey et al. (1978) exposed Sprague-Dawley rats (~85/sex/group) to 0, 600 or 1200 ppm styrene 6 hours/day, 5 days/week. The concentration in the high-dose group was reduced to 1000 ppm after 2 months due to observed narcosis in males. Exposure continued for

18.3 and 20.7 months for males and females, respectively (corresponding to the date when 50% mortality was reached in one test group of each sex). Surviving animals were sacrificed at 24 months. Interpretation of the tumor incidence data for males is difficult because of a high mortality rate in the control and 1000 ppm males groups due to murine pneumonia. In females, the incidence of mammary adenocarcinomas was significantly higher in the 600 ppm group than in the controls (7/85 compared with 1/85 in controls). The incidence in the control group, however, was low compared with historical controls. The incidences of combined leukemia-lymphosarcomas in the 0, 600 or 1000 ppm female group were 1/85, 6/85 and 6/85, respectively. The incidences in each of the treated groups were not statistically significantly different from concurrent controls, but were significantly different from historical controls. Pooling data from both exposed groups also yielded a significantly elevated tumor incidence compared with concurrent controls.

The carcinogenicity of styrene in humans has been assessed by studies of workers in the styrene-polystyrene manufacturing industry and the styrene butadiene synthetic rubber industry. Inhalation is the predominant route for occupational exposure. The available studies are limited by small cohort sizes, variable duration and intensity of exposure among the cohort members, inadequate exposure data and multiple chemical exposures of the workers. An elevated incidence of hematopoietic and lymphatic cancer has been reported for workers in the styrene-butadiene rubber industry (McMichael et al., 1976; Lemen and Young, 1976; Block, 1976; Ott et al., 1980), but these data are inadequate to indicate that styrene is a human carcinogen. The potential hazards of styrene-butadiene production were reviewed in a briefing on April 30, 1976 (NIOSH, 1976). IARC (1979) analyzed the reports submitted at the briefing and concluded that the data

were not sufficient to indicate an association between styrene or butadiene exposure and an increased risk of leukemia. A NIOSH-initiated retrospective cohort mortality study of two styrene-butadiene rubber plants (designated A and B) was described by Meinhardt et al. (1978, 1982). The cohort in plant A consisted of 1662 white males employed for ≥6 months (mean = 9.48 years) during 1943-1976. The plant B cohort comprised 1094 white males with a mean employment duration from 1950-1976 of 10.8 years. The measured exposure levels (which are not accurate measures of past exposure levels) were 0.94, 1.24 and 0.1 ppm for styrene, butadiene and benzene (a known leukemogenic agent), respectively, in plant A and 1.99 and 13.5 ppm for styrene and butadiene, respectively, in plant B. The SMR for lymphatic and hematopoietic malignancies in plant B was 78, explained by the healthy worker effect. The SMR was 155 for neoplasms of the lymphatic and hematopoietic tissue in plant A. The SMRs for a subgroup employed at plant A between 1943-1945 (exposed to higher styrene concentrations) were 212 for total lymphatic and hematopoietic malignancies and 278 for leukemia and aleukemia. These SMRs are not statistically significantly elevated based on the two-sided statistical test customarily used by NIOSH. The mortality for leukemia and aleukemia for the plant A subgroup was significantly increased at the p=0.05 level using the one-sided test, which the investigators felt was more appropriate.

McMichael et al. (1976) determined risk ratios of 6.2 for lymphatic and hematopoietic cancer, 3.9 for lymphatic leukemia and 2.2 for stomach cancer in a relatively small group of workers employed for >2 years in a styrene-butadiene rubber production plant. Smith and Ellis (1977) noted, however, that the relative risk for lymphatic and hematopoietic cancer was based on only four deaths. The SMRs determined for a Dow Chemical retrospective

cohort mortality study of employees involved in the development and manufacture of styrene products were 132 for lymphatic and hematopoietic cancer (excluding leukemia) and 176 for leukemia (Ott et al., 1980). Nicholson et al. (1978) and Frentzel-Beyme et al. (1978) did not find an elevated incidence of deaths due to cancer in two cohorts of styrene/polystyrene workers. An increase in the proportional mortality ratio for digestive system cancers in styrene/polystyrene workers was reported in an abstract (Liebling et al., 1982), but insufficient detail was provided to evaluate this finding.

5.2. MUTAGENICITY

Studies testing the mutagenic potential of styrene in the reverse mutation assay developed by Ames et al. (1973) are summarized in Table 5-1. Styrene did not increase the number of revertant colonies of Salmonella typhimurium in the absence of a mammalian metabolic activating system (see Table 5-1). When styrene was assayed in the presence of a metabolic activating system (S-9 fraction), there were mixed positive and negative results (see Table 5-1). Several investigators obtained negative results in both plate incorporation and spot test procedures using bacterial strains TA1535, TA1537, TA98 and TA100 (Stoltz and Withey, 1977; deFlora, 1981; Busk 1979; Loprieno et al., 1978). Styrene was tested at doses up to 1 mg/plate, which was toxic to the bacteria. De Meester et al. (1977, 1981) and Vainio et al. (1976) obtained negative results with bacterial strains sensitive to frameshift mutations, but positive results were observed using strains sensitive to base pair substitutions.

Several styrene metabolites have also been tested for mutagenic activity in bacteria. No mutagenic activity was observed when styrene glycol was assayed with and without metabolic activation in S. typhimurium strains TA1535, TA1537, TA1538, TA98 and TA100 (de Flora 1981; Vainio et al., 1976).

TABLE 5-1

Mutagenicity Assays of Styrene in Salmonella typhimurium

Exposure Method	Dose Level	Metabolic Activation[a]	Tester Strain	Response	Comments	Reference
Spot test	NR	+/-	TA1535 TA100 TA1537 TA1538 TA98	-/- -/- -/- -/- -/-	NC	Bush, 1979
Spot test	5 µg	-	TA1535 TA100 TA1537 TA1538 TA98	- - - - -	NC	Milvy and Garro, 1976
Plate incorporation	up to 5 µmol/plate (up to 0.52 mg/plate)	+/-	TA1535 TA1537 TA1538 TA98 TA100	-/- -/- -/- -/- -/-	The maximum dose tested was toxic.	de Flora, 1981
Plate incorporation	up to 1 mg/plate	+/-	TA1535 TA1537 TA1538 TA98 TA100	-/- -/- -/- -/- -/-	The assays were performed with both rat liver and hamster derived S-9. In addition to the plate incorporation assay, a spot test was conducted with similar negative results.	Stoltz and Withey, 1977
Plate incorporation	0.001-10 µmol/plate (0.0001-1.0 mg/plate)	+/-	TA1535	-/-	Metabolic activation was provided by liver preparations from male CD1 mice pretreated with phenobarbital.	Loprieno et al., 1978
Plate incorporation	0.001-15 µmol/plate (0.001-1.6 mg/plate)	+/-	TA1535 TA100	-/- -/-	At 15 µmol/plate, only 1% of the cells survived. The S-9 was prepared from both Aroclor 1254 and Clophen-C pretreated rats. Inhibitors of epoxide hydrase and glutathione conjugation were included in the assay.	Bush, 1979
Plate incorporation	1-15 µmol/plate (0.104-1.56 mg/plate)	+/-	TA1535 TA1537 TA1538 TA98 TA100	+/- -/- -/- -/- -/-	In the absence of S-9, the highest dose tested was 100 µmol/plate with ~10 times the spontaneous level reached at 11 µmol/plate. Survival at 11 µmol/plate was only 12%.	De Meester et al., 1977

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TABLE 5-1 (cont.)

Exposure Method	Dose Level	Metabolic Activation*	Tester Strain	Response	Comments	Reference
Plate incorporation	0.01-100 µmol/plate (0.001-10.4 mg/plate)	+/-	TA1535 TA1537 TA1538 TA100 TA98	+/- -/- -/- +/- -/-	Styrene produced a > doubling of the spontaneous rate at a single dose 10 and 1 µmol/plate for strains TA1535 and TA100, respectively. A more detailed study of TA1535 confirmed that styrene had a narrow effective range.	Vainio et al., 1976
Plate incorporation	10 µmol/plate	+	TA1535	+	Styrene was also studied by liquid incubation atmospheric exposure and the fluctuation test. Negative results were obtained with all but the plate incorporation test where there was approximately a 10-fold increase in revertant numbers.	Poncelet et al., 1980
Atmospheric exposure	NR	+	TA100 TA1535 TA1537 TA98	- - - -	Styrene was listed as 1 of 45 non-mutagenic compounds in this survey of the mutagenic potential of 71 chemicals identified in drinking water.	Simmon et al., 1977
Atmospheric exposure	24% (v/v) for 24 hours (240,000 ppm)	+/-	TA1538 TA1535 TA100 TA98 TA1538 TA1537 646	+/- +/- +/- -/- -/- -/- -/-	An ~2-4-fold increase over the spontaneous rate was observed in the responsive strains.	De Meester et al., 1981

*Provided by S-9 derived from rat liver unless otherwise specified in the comment.

NR = Not reported; NC = no comment

Mercapturic acid precursor metabolites of styrene were negative for mutagenicity in strain TA100 (Pagano et al., 1982). Styrene oxide was nonmutagenic when tested with bacterial strains sensitive to frameshift mutations, but has consistently been demonstrated to be mutagenic in strains TA1535 and TA100 both in the presence and absence of a mammalian metabolic activating system (De Meester et al., 1977, 1981; Busk, 1979, de Flora, 1981; Glatt et al., 1975; Loprieno et al., 1978; Milvy and Garro, 1976; Vainio et al., 1976; Drinkwater et al., 1978). A presumed metabolite of styrene, 1-vinylbenzene-3,4-oxide, was only weakly mutagenic in strain TA100 when tested by the preincubation procedure, but exhibted much greater mutagenic activity when 25% of the tested dose was added to the incubation mixture at 5-minute intervals (Watabe et al., 1982). This modified procedure was used because 1-vinylbenzene-3,4-oxide was unstable in aqueous buffer.

Since styrene demonstrated only limited mutagenic activity in S. typhimurium, but several intermediate metabolites of styrene were mutagenic, investigators have studied the metabolism of styrene as it relates to mutagenicity. Watabe et al. (1978a,b, 1979) proposed that the rapid metabolism of styrene oxide by epoxide hydratase might explain the limited mutagenic potential of styrene. Styrene was mutagenic when TCPO (3,3,3-trichloropropene oxide), an epoxide hydratase inhibitor, was added to the assay, but styrene oxide levels were not sufficient to account for the positive response, suggesting that another metabolite may be involved. The addition of glutathione to the assay system in the presence of a soluble supernatant fraction resulted in a substantial decrease in the mutagenic response of styrene oxide (Yoshikawa et al., 1980). These results were suggestive of a glutathione-S-transferase catalyzed interaction between styrene oxide and

glutathione. The results of El-Tantawy and Hammock (1980) led the investigators to conclude that the primary enzyme operating in the rapid inactivation of styrene oxide is epoxide hydratase rather than glutathione-S-transferase.

The results of mutagenicity assays of styrene in eukaryotic organisms are summarized in Table 5-2. The rate of forward mutations in the yeast, Schizosaccharomyces pombe, was not affected by styrene with or without metabolic activation (Bauer et al., 1980; Loprieno 1977, 1979). An increase in gene conversion occurred at the ade locus in Saccharomyces cerevisiae in a host-mediated assay, but the increase was not observed when purified microsomes from mouse livers provided the metabolic activation (Loprieno et al., 1976). There were mixed positive and negative results when styrene was assayed in mammalian cells in vitro. Direct incubation of styrene with V-79 cells resulted in negative responses without metabolic activation, and a marginal positive result in the presence of the S-9 fraction (Loprieno et al., 1976; Beije and Jenssen, 1982). When a liver perfusion system was used to provide metabolic activation, however, the mutation rate increased significantly (Beije and Jenssen, 1982). Styrene-treated Drosophila melanogaster exhibited a statistically significant increase in recessive lethal mutations compared with controls (Donner et al., 1979). The mutation frequencies were 0.60 and 0.09% in the styrene-exposed and control groups, respectively. The frequency in the styrene-exposed group was doubled by pretreatment with phenobarbitone, an inducer of drug-metabolizing enzymes.

A number of investigators have tested styrene for the ability to produce chromosomal damage. de Raat (1978) found no increase in the frequency of SCE in CHO cells incubated with styrene (50-100 µg/l) with or without metabolic activation. When cyclohexane oxide, an epoxide hydratase inhibitor, was added to the incubation mixture, the frequency of SCE increased.

TABLE 5-2

Mutagenicity Assays of Styrene in Eukaryotic Cells/Organisms

Test Organisms	Type of Assay	Exposure Method	Dose Level	Metabolic Activation	Response	Comments	Reference
S. pombe (P_1 strain)	forward mutation	liquid incubation	100 mM (10.4 mg/ml)	+/-	-/-	The metabolic activation was provided by purified microsomes from mouse liver.	Loprieno et al., 1976
S. pombe (P_1 strain)	forward mutation	liquid incubation	100 mM (10.4 mg/ml)	+	-	Fresh microsomes were added every 12 minutes during the 60-minute incubation period. Increased toxicity was observed, but no increase in mutagenicity occurred.	Bauer et al., 1980
S. pombe (P_1 strain)	forward mutation	host mediated (mouse)	1000 mg/kg by gavage	NA	-	The yeast were injected i.p. and reisolated 3, 6 or 12 hours after treatment.	Loprieno et al., 1976
S. cerevisiae (D_4)	gene conversion	host mediated (mouse)	1000 mg/kg by gavage	NA	+	The ade mutants appeared to increase with incubation time, with 1.03, 4.23, 4.75 and 5.38 mutants x 10^6 cells observed in cells isolated 0, 1, 3 and 6 hours after exposure.	Loprieno et al., 1976
V79 cells	forward mutation	1 hour in culture	8.5 or 17 mM (0.88 or 1.8 mg/ml)	-	-	Cells were examined for the ability to grow in the presence of 8-azaguanine.	Loprieno et al., 1976
V79 cells	forward mutation	4 hours in culture	240-960 μmol/4 ml (6.25-25.0 mg/ml)	+/-	-/-	Cells were examined for the ability to grow in the presence of 6-thioguanine. In the presence of S-9, there was a marginal positive response when the data from the three repli-experiments were combined.	Beije and Jenssen, 1982
V79 cells	forward mutation	4 hours in perfusate	240 and 480 μmol/ 100 ml of perfusion media (0.25-0.50 mg/ml)	+	+	Cells were examined for the ability to grow in the presence of 6-thioguanine. The metabolic activation was provided by an isolated perfused rat liver.	Beije and Jenssen, 1982
D. melanogaster	recessive lethal	in diet	200 ppm	NA	+	Induction of metabolism increased the frequency of mutations.	Donner et al., 1979

NA = Not applicable

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Styrene did not produce an increase in the frequency of chromosomal aberrations in CHL cells in the absence of metabolic activation, but chromosal aberrations increased when the cells were incubated with styrene and the S-9 fraction (Matsuoka et al., 1979; Ishidate and Yoshikawa, 1380). Chromosomal aberrations including chromatid gaps, breaks and exchanges, chromosomal exchange, ring formations and fragmentations occurred in 10-19% of the cells incubated with 0.25 mg styrene/mL and S-9 (Matsuoka et al., 1979). The mitotic index of human lymphocytes exhibited a dose-related decrease after in vitro incubation with styrene (Linnainma et al., 1978a,b). The mitotic index was similar to controls after a 72-hour incubation period using a styrene concentration of 0.008% (v/v), but at concentrations of 0.03 and 0.08% (v/v), the index decreased to 50 and 0%, respectively, of control values. Styrene produced chromosomal damage at a concentration of 0.03% (v/v) for 72 hours (Linnainmaa et al., 1978a,b). A dose-related increase in SCE was observed in cultured human lymphocytes incubated with styrene at concentrations of 0.5-5 mM (0.052-0.52 mg/mL) styrene (Norppa et al., 1980a).

Norppa et al. (1980b) exposed Chinese hamsters to styrene vapors at concentrations of 300 ppm, 6 hours/day, 4-5 days/week for 3 weeks. The frequency of chromosomal aberrations in bone marrow cells of exposed animals was similar to controls. In a similar study, male Wistar rats were exposed to 300 ppm styrene 6 hours/day, 5 days/week for 12 weeks (Meretoja et al., 1978a). After $\geq$9 weeks of exposure, the percentage of aberrant bone marrow cells increased to 8-12% compared with 1-6% in controls. Conner et al. (1979, 1980, 1982) exposed BDF_1 male mice to styrene concentrations of 104, 387, 591 and 922 ppm, 6 hours/day for 4 days. The incidence of SCE was elevated in bone marrow, alveolar macrophages and regenerating liver cells

at exposure levels of ≥387 ppm.. Reducing the number of days of exposure at the highest level (922 ppm) from 4 to 2 to 1 days resulted in a decrease in bone marrow SCE from 10.0 to 5.1 to 3.0/cell compared with 2.9/cell in controls. Similar responses were observed with the other two tissues. The investigators (Conner et al., 1979, 1980, 1982) suggested that the SCEs induced in extrahepatic tissues could not be explained by hepatic metabolism of styrene to styrene oxide because of their finding that partial hepatectomy did not affect the clastogenic response of bone marrow cells or alveolar macrophages.

Norppa (1981) injected male C57BL/6 mice intraperitoneally with styrene at dose levels of 250, 500, 1000 or 1500 mg/kg. The highest dose resulted in mortality in 2/4 animals. Statistically significant increases in micronuclei were observed only in the 250 and 1000 mg/kg group, and the magnitude of these increases was small. Micronuclei occurred in only 0.99% of the cells in the 1000 mg/kg group compared with 0.49% in the control.

An increase in chromosomal aberrations in peripheral lymphocytes has been reported in workers exposed to high levels of styrene (1-300 ppm) in the reinforced plastic and polyester resin industries (Meretoja et al., 1977, 1978b; Fleig and Theiss, 1978; Andersson et al., 1980; Camurri et al., 1983; Hogstedt et al., 1979). These occupational studies are characterized by multiple chemical exposures of workers and imprecise data regarding exposure levels.

The styrene metabolite, styrene oxide, exhibited mutagenic activity in most, but not all, eukaryotic test systems (Loprieno et al., 1976; Sugiura et al., 1979; Beije and Jenssen, 1982; Amacher and Turner, 1982; Donner et al., 1979). Styrene-oxide produced dose-related increases in SCE in Chinese hamster cells with metabolic activation (de Raat, 1978) and in isolated

human lymphocytes (Norppa et al., 1980a), but no cytogenetic damage has been induced after exposure of laboratory animals to styrene oxide (Fabry et al., 1978; McGregor, 1981; Norppa et al., 1979).

5.3. TERATOGENICITY

Teratologic evaluations of styrene have been conducted in rats by oral exposure and in mice, hamsters, rats and rabbits by inhalation exposure. In the oral study conducted by Dow Chemical Company, styrene was administered to pregnant Sprague-Dawley rats (29-30/group) by gavage at dose levels of 0, 90 or 150 mg/kg bw, 2 times/day on days 6-15 of gestation (Murray et al., 1976, 1978a). Maternal body weight gain and food consumption was significantly ($p<0.05$) reduced at both dose levels only on days 6-9 of gestation. Maternal mortality and percent of pregnancy were not significantly affected. No significant treatment-related effects were observed on mean incidences of implantations/dam, resorptions/litter or live fetuses/litter, or on mean fetal body weight or crown-rump length. No teratogenic effects were noted during gross external, soft tissue and skeletal examinations. To examine potential maternotoxic effects, an additional group of seven pregnant rats was dosed with 300 mg/kg/day of styrene on days 6-15 of gestation and necropsied on day 16 of gestation. Focal gastric ulceration indicated by small darkened areas in the stomach of 4/7 treated rats compared with 0/6 controls was the only sign of toxicity.

Investigators at Dow Chemical Company also administered styrene by inhalation to pregnant Sprague-Dawley rats (Murray et al., 1978a,b). Groups of 29-30 rats were exposed to 0, 300 or 600 ppm styrene 7 hours/day on days 6-15 of gestation. The only overt signs of possible maternotoxicity were significantly ($p<0.05$) reduced body weight gain and decreased food consumption on days 6-9 of gestation (but not days 10-15 or 16-20). There were no

significant differences in the number of live fetuses, resorptions/litter or mean fetal body weight. The average fetal crown-rump length was significantly ($p<0.05$) reduced in the 300 but not the 600 ppm group. The magnitude of the difference in length was small (43.8 mm at 300 ppm, compared with 44.8 mm for the concurrent controls). The incidence of skeletal variants including lumbar spurs and delayed ossification of sternebrae and vertebral centra were significantly higher only in the 300 ppm group. The incidences were, however, within the range observed for historical controls. The incidences of gross external and visceral malformations were not significantly different from controls.

Ragule (1974) also found no evidence for teratogenicity after exposing rats to 0.35, 1.2 or 12 ppm styrene, 4 hours/day throughout pregnancy. The incidence of resorptions was reportedly increased at these low-exposure concentrations. This study was available only as an abstract; details are insufficient to evaluate the results. Murray et al. (1978a) suggested that the results from the controls may not be representative, since Ragule (1974) reported the unusual finding of an absence of resportions in the control rats.

No signs of maternotoxicity, fetotoxicity or teratogenicity were noted in New Zealand rabbits exposed to 300 or 600 ppm styrene, 7 hours/day on days 6-16 of gestation (Murray et al., 1978a,b). Comparisons to controls included number of implantations/litter, number of live fetuses/litter, number of resorptions/litter, mean fetal body weight and crown-rump length, and gross external and visceral examination.

Kankaanpaa et al. (1980) exposed Chinese hamsters to 0, 300, 500, 750 or 1000 ppm styrene, 6 hours/day on days 6-18 of gestation. Teratogenicity was not observed at any exposure level. Fetotoxic effects were not reported at

exposure levels of ≤750 ppm, but a significantly ($p<0.001$) greater number of dead or resorbed fetuses was observed in the 1000 ppm group (33/50) compared with the controls (20/107). The number of live fetuses/litter at 1000 ppm (2.4) was not significantly different from the controls (5.3). Maternotoxicity was not reported.

The number of dead or resorbed fetuses was increased ($p<0.1$) in BNR mice exposed to 250 ppm styrene, 6 hours/day on days 6-16 of gestation compared with controls (Kankaanpaa et al., 1980). The incidence was 27% in exposed mice compared with 18% in controls. The number of live fetuses/litter was not significantly reduced. In preliminary experiments, a similar exposure to 500 ppm styrene resulted in deaths of 2/6 of the the pregnant mice and a fetal death rate of 47% among surviving mice. Exposure to 750 ppm resulted in deaths of 3/5 mice, and 19/20 fetuses in the surviving females were dead or resorbed. No teratogenic effects occurred in the 250 ppm group. The percentage of fetuses with minor skeletal abnormalities (rib fusions and extra ribs) was 2.9 in the 250 ppm group compared with 0.9 in the controls; this difference was not significant.

5.4. OTHER REPRODUCTIVE EFFECTS

Several Finnish investigators have examined potential human reproductive effects of occupational exposure to styrene. Hemminki et al. (1980) studied the frequency of spontaneous abortions among Finnish chemical workers. Data regarding spontaneous abortions, induced abortions and births for 1973-1976 were obtained from the Hospital Discharge Registry of the Finnish National Board of Health, and were matched by social security number to the membership registry of the Finnish Union of Chemical Workers. The rate of spontaneous abortion (number of spontaneous abortions x 100/number of pregnancies) and the ratio of spontaneous abortion (number of spontaneous abortions x

100/number of births) were both significantly ($p<0.01$) higher among a subgroup of styrene industry workers compared with all Finnish women. The analysis was based on only six spontaneous abortions among styrene industry workers.

Obstetric histories of 67 Finnish lamination workers exposed to styrene and 67 age-matched workers of similar social class with no obvious chemical exposure were compared by Harkonen and Holmberg (1982). The duration of exposure to styrene ranged from 0.5-10 years. Exposure levels were not measured, but a TWA styrene concentration of 66 ppm had been previously reported in the polyester plastics industry. The number of women with pregnancies or spontaneous abortions did not differ significantly between the styrene-exposed and the reference cohorts.

Mothers of 2/43? children born with CNS defects in Finland during a 9-month period were employed in the reinforced plastics industry, with regular exposure to styrene during pregnancy (Holmberg, 1977). The two defects were anencephaly and congenital hydrocephaly. Based on the Finnish national fertility rate for 1974, the authors estimated that the number of births expected in the cohort of reinforced plastic industry workers during the 9-month study would be ~12. The rate of anencephaly and hydrocephaly in the general population was reportedly 0.2/1000 live births and 0.3/1000 live births, respectively. The investigators noted that these estimates suggested a 300-fold increased rate of CNS malformations in babies of reinforced plastic industry workers compared with the general population (2/12 vs. 0.5/1000) during the 9-month study period. The results are based on a very small sample size; the low number of births precluded statistical analysis of the results.

5.5. CHRONIC AND SUBCHRONIC TOXICITY

5.5.1. Oral Exposure. Subchronic and chronic toxicity studies of styrene using the oral route of exposure are summarized in Table 5-3. Ponomarkov and Tomatis (1978) treated mice with 1350 mg styrene/kg, once a week. Treatments were discontinued after 16 weeks because of high early mortality among treated mice. Treated male and female groups experienced 50 and 20% mortality, respectively, at 20 weeks compared with ~100% survival for controls. Multiple centrilobular liver necrosis, hypoplasia of the spleen and severe lung congestion were the most common lesions in mice dying by 20 weeks. The liver was the most common site of lesions in treated animals dying by 20 weeks.

Agrawal et al. (1982) observed a significant ($p<0.01$) increase in the specific binding of ^{3}H-spiroperidol to dopamine receptors in the corpus striatum of rats dosed by gavage with styrene at levels of 200 or 400 mg/kg/day for 90 days. Corpus striatum weights and body weights were not significantly affected at either dose.

Srivastava et al. (1982) studied hepatic effects of styrene administered to rats by gavage at dose levels of 0 (vehicle control), 200 or 400 mg/kg/day, 6 days/week for 100 days. Body weight gain and liver weight were not significantly affected by exposure. Mitochondrial and microsomal enzyme assays of liver tissue indicated significant alterations in the activities of a number of drug-metabolizing enzymes. Benzo[a]pyrene hydroxylase and aminopyrene-N-demethylase activities exhibited significant dose-related increases, while glutathione-S-transferase activity decreased in a dose-dependent manner. Significant ($p<0.01$) decreases were observed in mitochondrial succinic dehydrogenase and β-glucuronidase activities at both dose levels, and in acid phosphatase activity at 400 mg/kg. At 400 mg/kg, mild

TABLE 5-3

Subchronic and Chronic Toxicity of Orally Administered Styrene

Species/Strain	Sex/Number	Age or Weight	Vehicle	Dose	Duration	Effect	Reference
Mice/B_{20}	male&female/ 84	weaned	olive oil	1350 mg/kg	1/week 16 weeks	High early mortality among treated mice. Liver was common site of lesions. Severe lung congestion and hypoplasia of spleen in some treated mice.	Ponomarkov and Tomatis, 1978
Rats/NR	male 6/group sacrificed for assay	8 weeks	peanut oil	0, 200, 400 mg/kg	90 days	No significant effect on body weight or striatal weight at either dose. Both doses resulted in a significant increase in the specific binding of ^{3}H-spiroperidol to dopamine receptors in the corpus striatum.	Agrawal et al., 1982
Rats/NR	male/NR	110 ± 5 g	ground nut oil	0, 200, 400 mg/kg	5 days/week 100 days	Activities of a number of drug-metabolizing enzymes in the liver were significantly altered at both doses. At 400 mg/kg, significant increases in SGOT and SGPT activities and focal necrosis in the liver. No effect on body or liver weights.	Srivastava et al., 1982
Rats/Wistar-derived	female 10/group 20 control	~2 months	olive oil/ gum arabic	0, 66.7, 133, 400, 667 mg/kg	5 days/week 6 months	At 66.7 and 133 mg/kg: no treatment-related effects observed; at 400 and 667 mg/kg: growth depression and increased liver and kidney weight, no histopathologic or hematologic effects.	Wolf et al., 1956
Rats/BD IV	21 pregnant rats plus 144 male/ offspring	weaned	olive oil	Single dose of 1350 mg/kg to dams on day 17. Weekly doses of 500 mg/kg to offspring after weaning.	1/week 120 weeks	Lesions of kidney and forestomach were common. Small necrotic foci in liver and lung, and kidney congestion in several animals that died at 50-60 weeks.	Ponomarkov and Tomatis, 1978
Dogs/beagle	NR	NR	peanut oil	0, 200, 400, 600 mg/kg	560 days	Minimal histopathologic effects in the liver, hematologic effects including increased numbers of Heinz bodies in erythrocytes and a decrease in packed cell volume at 400 and 600 µg/kg.	Quast et al., 1978

NR = Not reported

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liver damage was indicated by significantly ($p<0.02$) elevated SGOT and SGPT activities and focal necrosis (incidence not reported). Glucose-6-phosphatase activity in the liver was not affected by styrene administration.

Growth depression and increased liver and kidney weight occurred in rats dosed with 400 or 667 mg styrene/kg, 5 days/week for 6 months (Wolf et al., 1956). No histopathologic or hematologic effects were observed at these dose levels. No treatment-related effects were noted in rats dosed with 66.7 or 133 mg/kg, 5 days/week for 6 months.

Ponomarkov and Tomatis (1978) administered a single dose of 1350 mg styrene/kg to pregnant rats on day 17 of gestation. After weaning, the progeny were dosed with 500 mg/kg once a week for life. Surviving rats were sacrificed at 120 weeks. Body weight was not significantly affected by styrene administration. Several animals that died at 50-60 weeks exhibited small necrotic foci in liver parenchyma and moderate lung and kidney congestion. No liver damage was observed in animals that died after 80 weeks. Hyperplasia of the renal pelvis epithelium and forestomach lesions were common (incidences were not reported).

In a study by Quast et al. (1978), beagle dogs received styrene at dose levels of 0, 200, 400 or 600 mg/kg for 560 consecutive days. No treatment-related alterations in body weight, organ weights, urinalysis or clinical chemistry determinations (serum urea nitrogen, SGPT, SGOT and AP) were detected. No effects were reported at 200 mg/kg/day, with the possible exception of one dog that exhibited very slightly increased iron deposits in the liver and sporadic low-level occurrences of Heinz bodies in the red blood cells. At 400 and 600 mg/kg/day, packed red blood cell volume decreased, and there was a dose-related increase in the number of Heinz bodies in the red blood cells. Minimal histopathologic changes were

observed in the liver, including increased iron deposit in the reticuloendothelial cells at 400 and 600 mg/kg and increased numbers of intranuclear acidophilic crystalline inclusions in the hepatocytes at 600 mg/kg. No statistical comparisons were reported.

5.5.2. Inhalation Exposure. Table 5-4 summarized subchronic and chronic inhalation studies of styrene. A number of biochemical changes occurred in male rats exposed to 300 ppm styrene, 6 hours/day, 5 days/week for up to 11 weeks (Vainio et al., 1979). Lung glutathione levels were significantly ($p<0.05$) lower in exposed rats compared with controls after 2 and 4 weeks of exposure, but not after 6 and 11 weeks. Liver glutathione levels were significantly ($p<0.001$) decreased after 2 weeks of exposure; the depression was less marked after longer exposures. Hepatic cytochrome P-450 content and ethoxycoumarin deethylase activity increased significantly ($p<0.01$) after 2 weeks of exposure and remained elevated throughout the exposure period. Hepatic glucuronosyl transferase activity increased significantly by 6 weeks and epoxide hydratase activity increased by the end of the study. In the kidney, cytochrome P-450 content increased significantly ($p<0.05$) only after 11 weeks of exposure. Renal ethoxycoumarin deethylase activity increased significantly ($p<0.001$) after >2 weeks, while renal VDP glucuronosyl transferase activity significantly ($p<0.01$) increased after >6 weeks. Histologic changes in the liver, including parenchymal hydropic degeneration, steatosis and congestion occurred after 2 weeks of styrene exposure.

Seppalainen (1978) observed a transient increase in the motor conduction velocity of the tail nerve of rats exposed to 300 ppm styrene, 6 hours/day, 5 days/week for up to 11 weeks. The velocity was significantly ($p<0.05$) higher after 6 weeks, but not after 8 or 11 weeks of exposure.

TABLE 5-4

Chronic and Subchronic Inhalation Studies of Styrene

Species/Strain	Sex/Number	Age or Weight	Concentration (ppm)	Exposures	Duration	Effects	Reference
Rats/Wistar	M/40	adult	300	6 hours/day 5 days/week	77 days	Lung and liver glutathione levels depressed initially. Mixed function oxidase system enhanced after 2 weeks in liver and kidney. Other changes in hepatic and renal enzyme activities. Histologic liver alterations after >2 weeks.	Vainio et al., 1979
Rats/NR	NR/20 15 control	young adult	300	6 hours/day 5 days/week	77 days	Motor conduction velocity of tail nerve significantly higher ($p<0.05$) after 6 weeks, but not after 8 or 11 weeks.	Seppalainen, 1978
Rats/Wistar	M/40	adult	300	6 hours/day 5 days/week	77 days	Transient increase in serum creatine kinase activity and transient decrease in serum cholesterase. After >9 weeks, significant changes in enzyme activity in the brain, decreased protein content and increased RNA content in the brain.	Savolainen and Pfaffli, 1977
Rats/Wistar	M/26	adult	300	6 hours/day 5 days/week	28-119 days	No effect on behavior. Minimal effects on glial cells. Glial acid proteinase decreased significantly only at 4 weeks.	Savolainen et al., 1980
Rats/Wistar	M&F/50, 28	NR	1300, 2000	7-8 hours/day 5 days/week	148, 214 days	Eye and nose irritation at 1300 weight gain depression at 2000 ppm.	Spencer et al., 1942; Wolf et al., 1956
Guinea pigs/ heterogeneous	M&F/12-94	NR	650, 1300, 2000	7-8 hours/day 5 days/week	148-214 days	No effect at 650 ppm. 10% mortality after a few exposures to 1300 ppm due to acute lung irritation. Slow weight gain in survivors. No apparent effect on survival at 2000 ppm, but weight gain depressed.	Spencer et al., 1942; Wolf et al., 1956

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TABLE 5-4 (cont.)

Species/Strain	Sex/Number	Age or Weight	Concentration (ppm)	Exposures	Duration	Effects	Reference
Rabbits/ heterogeneous	M&F/12, 2	NR	1500, 2000	7-8 hours/day, 5 days/week	360, 148 days	No adverse effects reported.	Spencer et al., 1942; Wolf et al., 1956
Monkeys/rhesus	M&F/2	NR	1300	7-8 hours/day, 5 days/week	≤360 days	No adverse effects reported.	Spencer et al., 1942; Wolf et al., 1956
Rats/Sprague-Dawley	M/96/group	7-8 weeks	0, 600, 1000[a]	6 hours/day 5 days/week	18.3 months	High incidence of murine pneumonia in control and treated groups. Body weight depressed at 1000 ppm and for first 263 days at 600 ppm. Decreased absolute mean weights of liver and kidney at ≥1 measurement time for each concentration.	Jersey et al., 1978
Rats/Sprague-Dawley	F/96/group	7-8 weeks	0, 600, 1000[a]	6 hours/day 5 days/week	20.7 months	Mean liver weights and liver-to-body weight ratios significantly higher at some but not all measurement times for each concentration. Significantly reduced mean body weight at 1000 ppm. Several pathologic changes in the lungs at necropsy.	Jersey et al., 1978

[a]1200 ppm for first 2 months

NR = Not reported

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Savolainen and Pfaffli (1977) examined neurochemical effects of styrene by exposing male Wistar rats to 0 or 300 ppm styrene, 6 hours/day, 5 days/week for up to 11 weeks. Peak styrene levels in the brain and perinephric fat were reached after 4 weeks; levels declined after that time. Serum cholinesterase activity decreased significantly at 2 and 4 weeks, while serum creatine kinase activity increased significantly at 4 and 6 weeks. Activities of these two enzymes increased significantly in the brain only after 9 weeks of exposure. Other statistically significant biochemical changes in the brain observed after 9 or 11 weeks of exposure included increased acid proteinase activity and RNA content, and decreased protein content. Electrophoresis demonstrated minor alterations in the protein composition of spinal cord axons after 9 weeks.

To further study neurochemical effects, Savolainen et al. (1980) exposed male Wistar rats to 300 ppm styrene, 6 hours/day, 5 days/week for 4-17 weeks. The concentration of acid proteinase in glial cells was significantly ($p<0.05$) decreased after 4 weeks, but not after 8, 13 or 17 weeks of exposure. Glial glutathione concentration and glial NADPH-diaphorase activity were not significantly altered. Savolainen et al. (1980) noted that the minimal effect of styrene on glial cells was interesting, since protein destruction was observed in whole brain homogenate after 9 weeks of exposure to styrene (Savolainen and Pfaffli, 1977). Behavior, including ambulation, rearing, preening frequency and preening time, was not significantly altered (Savolainen et al., 1980).

Dow Chemical Company conducted subchronic inhalation studies of styrene using Wistar rats, albino guinea pigs, albino rabbits and rhesus monkeys (Spencer et al., 1942; Wolf et al., 1956). Animals were exposed 7-8 hours/day, 5 days/week. Eye/nose irritation was observed in rats exposed to

1300 ppm styrene for 7 months, but no effects were observed in body weight, organ weights, gross or histopathology, or hematology. Weight gain depression was observed in rats exposed to 2000 ppm for 5 months; no pathologic or hematologic examinations were conducted. Guinea pigs were exposed to 650, 1300 or 2000 ppm for 6, 7 or 5 months, respectively. Body weight gain, organ weights and gross pathology were not altered at the lowest concentration. About 10% mortality occurred in guinea pigs after a few exposures to 1300 ppm, apparently due to severe lung irritation. Surviving animals gained weight slowly. At 2000 ppm, guinea pigs exhibited depressed body weight gain, but survival was not affected. Rabbits exposed to 1300 ppm for up to 1 year showed no treatment-related effects on weight gain, gross or histopathology, or hematology. No effects on weight gain or appearance were noted in rabbits exposed to 2000 ppm for 5 months. No evidence of irritation, intoxication, gross or histopathologic effects, or hematologic effects was observed in two male and two female monkeys exposed to 1300 ppm styrene for 7 or 12 months, respectively.

In a chronic inhalation study, Jersey et al. (1978) exposed male and female Sprague-Dawley rats to 0, 600 or 1000 ppm styrene, 6 hours/day, 5 days/week. The high concentration was reduced from 1200 to 1000 ppm after 2 months because of overt narcosis in males. Exposures were terminated when mortality reached 50% in one group of each sex; surviving animals were sacrificed at 24 months. The results for males are difficult to interpret because of a high incidence of murine pneumonia. Mean body weight was significantly decreased in treated males for the first 263 days; after that, the mean body weight in the 600 ppm group was similar to controls. The absolute kidney and liver weights were decreased significantly at 6 and 12 months, respectively, in the 600 ppm group. The kidney and liver weight changes were significant at both 6 and 24 months for the 1000 ppm group.

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A minimal decrease in the cytoplasmic microvesiculation of the hepatocytes was observed at 6 and 12 months. No significant differences were observed in hematology, urinalysis and clinical chemistry results, and no exposure-related histopathologic changes were noted at necropsy. In females, mean body weight was significantly depressed in the high-dose group for the first 506 days. Mean liver weights and liver/body weight ratios were significantly higher at 6 and 12 months in the 600 ppm group and at 6 and 24 months in the 1000 ppm group. Hematology, urinalysis and clinical chemistry results did not reveal any exposure-related effects in females. Lung effects, including small subpleural pale foci at both exposures and focal alveolar histiocytosis at 1000 ppm, were noted at the termination of the study, but no other gross or histopathologic changes were considered exposure-related.

5.5.3. Occupational Exposure. Numerous studies have been conducted to assess the health effects in humans of occupational exposure to styrene vapors. Many of the occupational studies are limited by lack of precise exposure data, lack of controls, grouping of individuals with widely differing exposure durations, concurrent exposure to multiple chemicals and cross-sectional designs.

Neurophysiological and behavioral effects of occupational exposure to styrene are summarized in Table 5-5. The following discussion highlights the results of a few of these studies. Mean simple reaction times were significantly longer in six subjects exposed to >150 ppm TWA styrene for up to 12 years, but not in 11 workers exposed to <150 ppm TWA styrene compared with their respective age-matched controls (Gotell et al., 1972). Gamberale et al. (1975) reported increased reaction times in workers exposed to 13-101 ppm styrene. Sensory neuropathy was suggested by significantly longer

TABLE 5-5

Behavioral and Neurophysiological Effects of Occupational Styrene Exposure (Inhalation)

Study Population	Control Population	Exposure Duration	Exposure Level	Effect	Reference
17 Swedish male reinforced plastic workers; median age = 28	17 age-matched subjects from motor workshop	few days to ~12 years	>150 ppm (high) <150 ppm (low) TWA	The 6 subjects in the high exposure groups had significantly ($p<0.01$) longer mean simple reaction times when measured both before and after work compared to their controls. No difference in reflexes, vibration sensitivity or Romberg test in either group.	Gotell et al., 1972
186 Swedish boat manufacturers	NC	0.1-11 years, mean = 2.7	13-101 ppm mean concentration	Longer and more irregular reaction times in exposed workers; effects were still evident in the morning (after a nights rest). Further details of results not available.	Gamberale et al., 1975
7 Swedish boat manufacturers	NC	mean 10 ± 7 years	9 ± 4 ppm[a] mean concentration	Exposed workers had longer reaction times in afternoon than in morning prior to work, whereas afternoon reaction times were shorter in controls. No effect on tests (unspecified) of sensory and motor functions.	Kjellberg et al., 1979
27 British fiberglass boat hull manufacturers; mean age = 23	27 unexposed workers from same factory; mean age = 26	NR	11-191 ppm TWA mean = 92 ppm	Five objective tests used (simple reaction time, vigilance task, digit-symbol substitution, forward and backward digit-span tests). The only significant ($p<0.05$) effect was a slower mean reaction time in exposed workers in the morning after a weekend of no occupational exposure. At the end of the work shift reaction time was similar to controls.	Cherry et al., 1980
81 West German styrene-exposed workers	34 unexposed workers	NR	NR	Significant effects of exposure intensity noted on reasoning, word fluency, substitution, cancellation, perceptual accuracy, interference, digit reproduction and choice reaction. Significant effects of exposure duration noted on digit reproduction, interference, perceptual accuracy and cancellation tests. Further details not reported.	Seeber et al., 1979

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TABLE 5-5 (cont.)

Study Population	Control Population	Exposure Duration	Exposure Level	Effect	Reference
98 Finnish male laminating workers; mean age = 30	43 male concrete re-inforcement workers; mean age = 33	0.5-14 years, mean = 4.9	7-674 mg/l[b] (low) or 1762-4715 mg/l (high)	Ten tests measuring general intelligence, visuomotor speed, visuomotor accuracy, memory, vigilance, psychomotor performance and personality performed. Only 2 variables differed significantly ($p<0.05$) between exposed and control: poorer visuomotor accuracy and longer latency time in Rorschach test in exposed workers. No significant difference in reaction time. Workers with high levels of urinary mandelic acid had significantly ($p<0.05$) poorer visuomotor accuracy and psychomotor performance than low exposure group.	Lindstrom et al., 1976
				Change in visuomotor accuracy first noticeable when urinary mandelic acid concentration >800 mg/l (~36 ppm 8-hour estimated TWA). More pronounced decrement in visuomotor and psychomotor performance at an estimated 8-hour TWA of >55 ppm (mean mandelic acid concentration >1200 mg/l).	Harkonen et al., 1978
				Personality reactions evaluated using 20 Rorschach variables. Fewer emotional reactions, lower anxiety and a lower number of neurotic signs in exposed group.	Lindstrom and Hartelin, 1980
96 of 98 Finnish workers described above	none	0.5-14 years, mean = 5	7-4715 mg/l[b]	23/96 (24%) of workers had abnormal EEGs; significantly ($p<0.01$) higher than expected for general population (10%). Subjects with abnormal EEGs had significantly ($p<0.05$) higher mandelic acid concentrations than subjects with normal EEGs. Proportion of abnormal EEGs was normal among workers with mandelic acid concentrations <700 mg/l (4/38, 10%), but was higher among workers with higher styrene exposure (19/58, 33%). Exposure duration did not affect proportion of abnormal EEGs, but longer-exposed workers had lower mandelic acid concentrations.	Seppalainen and Harkonen, 1976
				A marked change in proportion of normal and abnormal EEGs when mandelic acid concentrations were >700 mg/l (31 ppm estimated 8-hour TWA). Proportion of abnormal EEGs reached a plateau at 800 mg/l.	Harkonen et al., 1978

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TABLE 5-5 (cont.)

Study Population	Control Population	Exposure Duration	Exposure Level	Effect	Reference
40 of 96 Finnish workers described above; mean age = 30	30 males; mean age = 30	NR	30-3811 mg/m³[b]	No significant differences found in nerve conduction velocity tests (maximal motor conduction velocity of median, ulnar, deep peroneal and posterior tibial nerves, conduction velocity of slower motor fibers of ulnar and deep peroneal nerves, sensory conduction velocity of median and ulnar nerves).	Seppalainen and Harkonen, 1976
13 male polyester resin workers; mean age = 44	6 male hospital transportation service workers; mean age = 36	1-12 years	125 ppm TMA (range 74-175 ppm)	Incidence of several subjective symptoms (tiredness, reduced short-term memory and giddiness) exposure-related based on questionnaire results. Nerve conduction measurements: mean duration of SAPs at the wrist significantly ($p<0.05$) longer for the 125 and 5 ppm groups compared with controls. Amplitude of the SAPs significantly ($p<0.05$) lower in the 47 ppm group. No significant difference between groups in motor conduction velocity (of median, fibular and posterior tibial nerves) or distal motor latency of median nerve.	Rosen et al., 1978
10 male polyester resin production workers; mean age = 43		2-14 years	47 ppm TMA (range 21-67 ppm)		
10 male polystyrene production workers; mean age = 44		5-15 years	<5 ppm TMA[c]		
18 plastic industry workers	none	mean 1.9 years	43-130 ppm	13 (72%) workers had abnormal EEG findings.	Klimkova-Deutschova, 1962
14 female styrene-exposed workers	none	NR	NR	9 (64%) workers had abnormal EEG findings; occurrence of theta waves was the most frequent abnormality.	Roth and Klimkova-Deutschova, 1963
412 styrene production/polymerization plant workers	none	<32 years	<1 ppm or ~5-20 ppm TMA	Distal hypothesis (touch and pain) observed in 24 cases (6%), hyperactive deep tendon reflexes observed in 15 cases (4%) and decreased deep tender reflexes observed in 13 cases (3%). Radial nerve conduction velocities slow (<55 m/sec) in 15/80 cases; peroneal nerve conduction velocities slow (<40 m/sec) in 12/73 cases. No comparison to control or general population.	Lilis et al., 1978

[a]Concurrent acetone exposure (35 ± 19 ppm mean concentration)

[b]Measured as mean urinary mandelic acid concentration

[c]Concurrent isopentane exposure in some workers ("minor" significance)

NC = Not characterized; NR = not reported

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duration or reduced amplitude of sensory action potentials at the wrist of workers exposed to a range of styrene concentrations (Rosen et al., 1978). Motor conduction velocity was not affected. Seppalainen and Harkonen (1976) did not find any significant differences in nerve conduction tests between a styrene-exposed cohort and a control group.

A number of tests have been conducted on a cohort of 98 Finnish male laminating workers exposed to a wide range of styrene concentrations (see Table 5-5). Urinary mandelic acid levels were used to estimate styrene exposure. Ten tests measuring intelligence, memory, vigilance, visuomotor speed and accuracy, psychomotor performance and personality were administered ~20 hours after the last styrene exposure (Lindstrom et al., 1976). Visuomotor accuracy was significantly poorer and the latency time in the Rorschach test was significantly longer in the cohort of styrene-exposed workers with urinary mandelic acid levels ranging from 7-4715 mg/L, than in 43 unexposed concrete reinforcement workers with a similar age distribution and educational level. Visuomotor accuracy and psychomotor performance were significantly ($p<0.005$) poorer in a subgroup of workers with high levels of mandelic acid (1762-4715 mg/L) compared with a subgroup with lower exposure (urinary mandelic acid ranging from 7-674 mg/L). The relationship between styrene exposure and neurologic function was analyzed by Harkonen et al. (1978). Chi-square analysis indicated that the incidence of reduced visuomotor accuracy increased significantly ($p<0.05$) when the mean mandelic acid concentration was >800 mg/L. At >1200 mg/L, a more pronounced decrease in both visuomotor and psychomotor performance occurred. The proportion of abnormal EEGs (measured in 96 of 98 members of the cohort) increased markedly when the mandelic acid concentration was $\geq$700 mg/L.

Using a regression equation previously estimated by the research group (Engstrom et al., 1976) to convert mandelic acid concentrations to 8-hour TWA styrene concentrations, resulted in estimates of 36 ppm TWA styrene for the deterioration in psychological function threshold and 31 ppm TWA for the increased incidence of abnormal EEGs threshold.

Investigators have also studied hepatic, renal, hematologic and respiratory effects of styrene exposure in humans. A detailed review of these studies can be found in Neal et al. (1983). The activities of SGOT, SGPT and GGT (indicators of hepatotoxic effects) were not significantly different from controls in 84 styrene workers exposed to <1 ppm styrene (Thiess and Friedheim, 1978) and were normal in 101 workers exposed to an average of 23-70 ppm styrene (Chmielewski and Hac, 1976). Levels of SGOT and SGPT were significantly higher, however, in 35 Swedish laminating workers exposed to <100 ppm TWA styrene compared with controls (Axelson and Gustavson, 1978). Lorimer et al. (1976, 1978) found a significantly higher incidence of abnormal GGT in workers exposed to 5-20 ppm styrene than in workers exposed to <1 ppm. Holtz et al. (1980) also reported a tendency for higher serum enzyme activities in individuals exposed to 50-100 ppm compared with individuals exposed to <50 ppm styrene.

Askergren et al. (1981a,b) found that the mean urinary concentration of albumin was significantly higher in styrene-exposed workers (exposure level not clearly defined) than in controls, but indicators of renal tubular damage were negative. Glomerular filtration rate was not altered in the styrene-exposed group (Askergren et al., 1981c). Lorimer et al. (1976, 1978) found significant differences with respect to acute lower respiratory symptoms between high (5-20 ppm) and low (<1 ppm) exposure groups in a survey of 488 styrene-exposed workers. Harkonen (1977) and Axelson and

Gustavson (1978) reported no marked differences in lung function test results of styrene-exposed workers. Obstructive lung changes were noted in 4/21 styrene-exposed workers examined by Chmielewski and Renke (1975), but no comparison was made with controls. Platelet count was significantly lower in a group of 21 workers exposed to ~70 ppm styrene for an average of 10 years compared with 101 workers exposed to 23-70 ppm for an average of 1 year (Chmielewski and Renke, 1976). A number of other hematology parameters differed between the group exposed to ~70 ppm styrene and 20 unexposed controls. Results of hematology analyses have been reported by other investigators (Lorimer et al., 1976, 1978; Thiess and Friedheim, 1978; Checkoway and Williams, 1982), but lack of comparison with controls and/or low exposure levels makes interpretation of their results difficult.

5.6. OTHER RELEVANT INFORMATION

An acute oral LD_{50} value for styrene of ~5000 mg/kg was reported for rats treated by gavage (Wolf et al., 1956). LC_{50} values for rats ranged from 2700 ppm x 4 hours (Jaeger et al., 1974) to 4620 ppm x 6 hours (Bonnet et al., 1982), while LC_{50} values reported for mice are 4930 ppm x 4 hours (Shugaev, 1969) and 2430 ppm x 6 hours (Bonnet et al., 1982).

In controlled experiments with human volunteers, Stewart et al. (1968) found that inhalation exposure to 1600 mg/m³ (376 ppm) styrene for 1 hour caused eye and throat irritation and altered neurophysiological function (reduced performance in the Crawford Manual Dexterity Collar and Pin test, the modified Romberg test and the Flannagan Coordination test). No overt signs of toxicity were observed during or after exposure to 497 mg/m³ (117 ppm) for 2 hours, while exposure to 921 mg/m³ (216 ppm) resulted in nasal

irritation after 20 minutes (Stewart et al., 1968). Exposure to 3407 mg/m³ (800 ppm) for 4 hours resulted in eye and throat irritation, drowsiness, listlessness and unsteadiness (Carpenter et al., 1944). Reaction time increased significantly ($p<0.05$) during the last of four consecutive 30-minute exposures to increasing styrene concentrations (50, 150, 250 and 350 ppm) (Gamberale and Hultengren, 1974).

Wolf et al (1956) reported an odor threshold for styrene of 42.6 mg/m³ (10 ppm). Alexander et al. (1982) determined an average odor threshold of 3.6 mg/m³ for styrene and an average taste threshold of 0.12 mg/l (0.12 ppm) in 40°C water.

6. AQUATIC TOXICITY

6.1. ACUTE EFFECTS

The acute lethal levels of styrene have been determined for several species of warm water fish (Table 6-1). Pickering and Henderson (1966) reported 24-, 48- and 96-hour LC_{50} values for bluegills, fathead minnows, goldfish and guppies. All tests were conducted under static conditions and all species were tested in soft water (20 mg $CaCO_3$/ℓ). Tests with fathead minnows were conducted in hard (360 mg $CaCO_3$/ℓ) and soft water for comparative purposes; styrene appeared slightly more toxic in soft water. The most sensitive freshwater species tested was the bluegill sunfish, with a 96-hour LC_{50} value of 25.05 mg/ℓ. Bridie et al. (1979) reported a 24-hour LC_{50} value for goldfish of 26.0 mg styrene/ℓ, which differed from the level (64.74 mg/ℓ) reported by Pickering and Henderson (1966) for the same species. The sheepshead minnow, a marine species, appeared notably more sensitive to sytrene, having a 96-hour LC_{50} value of 9.1 mg/ℓ (Heitmuller et al., 1981). The "no observed effect concentration" for sytrene in the sheepshead minnow was 5.1 mg/ℓ, as determined in 96-hour tests.

In the water flea, Daphnia magna, median lethal levels of styrene were 27 and 23 mg/ℓ, respectively, in tests of 24- and 48-hour durations (Le Blanc, 1980). The concentration having no discernible effects was <6.8 mg/ℓ. In the brine shrimp, Artemia salina, 24- and 48-hour LC_{50} values were 68 and 52 mg styrene/ℓ, respectively (Price et al., 1974). Lindstrom and Lindstrum (1980) reported that the amphipod, Pontoporeia affinis, could survive only a few hours of exposure at 69 mg styrene/ℓ.

6.2. CHRONIC EFFECTS

Pertinent data regarding the effects of chronic exposure to styrene in aquatic organisms were not located in the available literature.

TABLE 6-1

Acute Lethal Effects of Styrene to Fish

Species	Duration (hours)	Median Lethal Concentration (mg/ℓ)[a]	Reference
Bluegill sunfish, *Lepomis macrochirus*	24	25.05	Pickering and Henderson, 1966
	48	25.05	
	96	25.05	
Fathead minnow, *Pimephales promelas*	24	56.73[b]	Pickering and Henderson, 1966
	48	53.58[b]	
	96	46.41[b]	
Fathead minnow; *P. promelas*	24	62.81[c]	Pickering and Henderson, 1966
	48	62.81[c]	
	96	59.30[c]	
Goldfish, *Carassius auratus*	24	64.74	Pickering and Henderson, 1966
	48	64.74	
	96	64.74	
Goldfish, *C. auratus*	24	26.0	Bridie et al., 1979b
Guppies, *Lebistes reticulatus*	24	74.83	Pickering and Henderson, 1966
	48	74.83	
	96	74.83	
Sheepshead minnow, *Cyprinodon variegatus*	24	9.1	Heitmuller et al., 1981
	48	9.1	
	72	9.1	
	96	9.1	

[a]All tests were conducted under static conditions.

[b]Soft water conditions (20 mg $CaCO_3$)

[c]Hard water conditions (360 mg $CaCO^3$/ℓ)

6.3. PLANT EFFECTS

Using the inhibition of cell multiplication as a measure of toxicity, Bringmann and Kuehn (1978, 1980) reported effective concentrations of styrene in the blue-green alga, Microcystis aeruginosa, and green alga, Scenedesmus quadricauda. The blue-green was more sensitive with effective toxic levels at 67 mg/l, while styrene at 200 mg/l was not inhibitive of growth in S. quadricauda.

6.4. RESIDUES

Price et al. (1974) reported that styrene was 65, 78 and 87% biodegraded, by oxidation, after 10, 15 and 20 days in freshwater. In saltwater, styrene concentration was decreased by 8, 12, 21 and 80% after 5, 10, 15 and 20 days, respectively.

6.5. OTHER RELEVANT INFORMATION

The swimming activity of the amphipod, Pontoporeia affinis, was stimulated by styrene at concentrations between 2.3 and 23 mg/l. Higher styrene levels (35 and 46 mg/l) caused amphipods to cease swimming for several days, then resume greater than normal activity (Lindstrom and Lindstrum, 1980)

Volatilization of styrene from the test chambers was high in experiments by Lindstrom and Lindstrum (1980), and may have been neglected in other toxicity tests.

7. EXISTING GUIDELINES AND STANDARDS

7.1. HUMAN

NIOSH (1983) recommends that occupational exposure to styrene be controlled to a concentration limit of 50 ppm TWA for up to a 10-hour day, 40-hour workweek, with a ceiling limit of 100 ppm (based on a 15-minute sampling period). The ACGIH (1983) curently recommends a TLV-TWA for styrene in workroom air of 50 ppm, with a STEL of 100 ppm. The TLV was reduced in 1981 from the previous limit of 100 ppm (ACGIH, 1981). The health standard for styrene exposure recommended by OSHA (Code of Federal Regulations, 1981) is an 8-hour TWA concentration of 100 ppm, a ceiling concentration of 200 ppm, and a maximum peak of 600 ppm for $\leq$5 minutes in any 3-hour period.

7.2. AQUATIC

Guidelines and standards for the protection of aquatic organisms from the toxic effects of styrene were not located in the available literature.

8. RISK ASSESSMENT

8.1. Quantification of Non-Carcinogenic Effects

The available studies have not demonstrated conclusively that styrene is carcinogenic. Styrene has been tested for mutagenicity in a variety of systems (see Section 5.2.). Styrene was negative for mutagenicity in Salmonella typhimurium without a mammalian metabolic activation system; in the presence of metabolic activation, there were mixed positive and negative results. Styrene was mutagenic in eukaryotic organisms including yeast, cultured mammalian cells and fruit flies in the presence of metabolic activation. The metabolite, styrene oxide, has been demonstrated to be mutagenic in prokaryotic and eukaryotic cells in vitro. The incidence of chromosomal aberrations increased in cultured mammalian cells and isolated human lymphocytes exposed to styrene and in cells of rats and mice after in vivo exposure to styrene vapors. These results suggest that the active genotoxic agent is a metabolite of styrene.

The results of the chronic animal bioassays are inconclusive even when viewed collectively. Ponomarkov and Tomatis (1978) found no statistically significant increases in any tumor types in C57BL mice and BD IV rats administered styrene by gavage. In the same study, there was a statistically significant increase in lung adenomas and carcinomas in O_{20} mice dosed weekly with 1350 mg styrene/kg. This dose level resulted in severe toxic effects and early mortality; these tumors were common to this strain of mice. The investigators did not consider the evidence adequate to designate styrene as a carcinogen. In the NTP (1979) bioassay, no statistically significant increase in tumors was found for Fischer 344 rats or female $B6C3F_1$ mice exposed to styrene by gavage. An increased incidence of

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alveolar/bronchiolar carcinomas and adenomas occurred in male mice dosed with 300 mg/kg; this incidence was significantly higher compared with vehicle controls but not historic controls. The incidence of brain tumors was not elevated in Sprague-Dawley rats exposed to styrene by gavage or inhalation (Maltoni et al., 1982). In a chronic inhalation study using Sprague-Dawley rats, high mortality due to murine pneumonia precluded evaluation of data for males. There was a statistically significant increase in mammary adenocarcinomas in the lower exposure group of females when compared with concurrent, but not historic, controls. The incidence of leukemia-lymphosarcomas was significantly increased at both exposure levels only when compared with historic controls (not concurrent controls). Occupational studies have not completely clarified the carcinogenic potential of styrene in humans (see Section 5.1.). These studies are limited by multiple chemical exposures of the cohort and/or small cohort sizes.

Styrene was not teratogenic in rats following oral exposure or in rats, mice, rabbits and hamsters using the inhalation route of exposure (Murray et al., 1976, 1978a,b; Kankaanpaa et al., 1980). Kankaanpaa et al. (1980) observed fetotoxicity in Chinese hamsters exposed to 1000 ppm styrene (6 hours/day, days 6-18 of gestation), and in BNR mice similarly exposed to 250 ppm styrene. It was not reported whether these exposures were toxic to the dams. A reported association between spontaneous abortions and styrene exposure in Finnish chemical workers (Hemminki et al., 1980) is difficult to interpret because of the small sample size, variable exposure levels and multiple chemical exposures, and it is not supported by the results of Harkonen and Holmberg (1982).

In an oral chronic bioassay, adverse effects were reported in rats following administration of styrene by gavage at a dose level of 500 mg/kg once a week (Ponomarkov and Tomatis, 1978). This study is not appropriate for risk assessment, because the dose schedule (weekly) would likely result in wide fluctuations of styrene concentrations in the body. Based on the pharmacokinetic data, styrene is apparently rapidly metabolized and eliminated from the body (see Chapter 4). No other chronic oral toxicity studies are available.

Agrawal et al. (1982) found a statistically significant ($p<0.01$) increase in the specific binding of ^{3}H-spiroperidol to corpus striatal membranes in rats receiving daily doses of 200 or 400 mg styrene/kg for 90 days. The increased sensitivity did not appear to be dose-related. Body weight and striatal weight were not affected at these doses. This biochemical effect in the brain suggests a subchronic NOAEL of 400 mg/kg. Dose-dependent changes in the activities of a number of drug-metabolizing enzymes occurred when rats were given styrene orally at dose levels of 200 or 400 mg/kg, 6 days/week for 100 days (Srivastava et al., 1982). The levels of SGOT and SGPT were elevated over controls at both doses, but the difference was significant ($p<0.02$) only at 400 mg/kg. Areas of focal necrosis in the liver were observed at 400 mg/kg only. Body weight gain and liver weight were not significantly different between treated and control rats. This study defines a subchronic NOAEL of 200 mg/kg, 6 days/week (171 mg/kg/day, 7 days/week) and a LOAEL of 400 mg/kg, 6 days/week (343 mg/kg/day, 7 days/week). Wolf et al. (1956) found no body weight, organ weight or pathological effects in rats administered oral doses of styrene at levels of 66.7 or 133 mg/kg, 5 days/week for 6 months. No hematologic or histopathologic effects were observed at doses of 400 or 667 mg/kg, but growth rates were

depressed and liver and kidney weights were increased at both doses. A subchronic NOAEL of 133 mg/kg, 5 days/week (95 mg/kg/day, 7 days/week) and a LOAEL of 400 mg/kg, 5 days/week (286 mg/kg/day, 7 days/week) can be derived from this study. No adverse effects were observed in dogs administered styrene by gavage at a dose of 200 mg/kg/day, 7 days/week for 560 days (Quast et al., 1978). There were minimal histopathologic changes in the liver, increased number of Heinz bodies in red blood cells and a decreased packed cell volume in dogs exposed to 400 or 600 mg/kg. Based on this study, available only as an abstract, a dog subchronic NOAEL for styrene is 200 mg/kg/day and a LOAEL is 400 mg/kg/day.

The NOAEL of 200 mg/kg/day defined by the subchronic study in dogs (Quast et al., 1978) is the highest NOAEL that is less than any reported LOAELs. The biological endpoints considered in this study were body weight, organ weight, urinalysis and hematology parameters. This NOAEL is supported by the subchronic studies in rats, which defined NOAELs of 400 mg/kg/day based on neurochemical effects (Agrawal et al., 1982), 171 mg/kg/day based on enzyme induction and other hepatic effects (Srivastava et al., 1982) and 95 mg/kg/day based on body weight, organ weights, hematology and histopathology (Wolf et al., 1956). Uncertainty factors of 10 for both intra- and interspecies variability to the toxicity of styrene and an additional uncertainty factor of 10 due to the relatively short duration of the study yield an overall uncertainty factor of 1000. No ADI for styrene is calculated due to its carcinogenic effects.

8.2. Quantification of Carcinogenic Effects

Data on the increased incidence of lung tumors (adenoma and carcinoma) in O_{20} strain mice (Ponomarkov and Tomatis, 1978), shown in Appendix R,

can be used for quantitative assessment of cancer risk if the assumption is made that styrene may have carcinogenic potential. There is only limited evidence to support this assumption. Based on the Ponomarkov and Tomatis data and using a linearized multistage model adopted by the U.S. EPA (1980), a carcinogenic potency factor (q_1^*) for humans of 1.34 $(mg/kg/day)^{-1}$ was calculated from the data for male mice and a q_1^* of 2.47 $(mg/kg/day)^{-1}$ was calculated from the data for female mice. Because the multistage model cannot accommodate a tumor incidence of 100% when only a single dose is tested, the tumor response for female mice was adjusted from 32/32 to 31/32 and the transformed dose reduced by multiplying the calculated transformed dose, 25.7 mg/kg/day, by the ratio 31/32 to arrive at an adjusted transformed dose of 24.9 mg/kg/day. The higher of the two q_1^* values is the appropriate basis for the estimation of cancer risk levels. The doses corresponding to increased lifetime cancer risks for a 70 kg man of 10^{-4}, 10^{-5} and 10^{-6} are 2.83×10^{-3}, 2.83×10^{-4} and 2.83×10^{-5} mg/day, respectively. These criteria, which reflect lifetime exposure, are not completely conclusive in light of the short exposure duration (13% of lifetime) and the small number of animals in each dose group.

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APPENDIX

LITERATURE SEARCHED

This profile is based on data identified by computerized literature searches of:

CA SEARCH (Files 308, 309, 310, 311, 320)
TOXLINE
MEDLINE
RTECS
SCI SEARCH
OHM TADS
STORET
SRC Environmental Fate Data Bases
SANSS
AQUIRE

These searches were conducted in July, 1983 and updated in December, 1983. In addition, hand searches were made of Chemical Abstracts (Collective Indices 6 and 7th), and the following secondary sources were reviewed:

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Part of Section 3.3. of this document was adapted from a report prepared by Syracuse Research Corp. for the U.S. EPA and listed in the reference list

of this profile as Santodonato et al. (1980). Some of the summary tables in Chapter 5 of this document were adapted from the following report:

Hansen, B.C., D.A. Gray and P.R. Durkin. 1984. External Review Draft of Drinking Water Criteria Document on Styrene. Prepared by Syracuse Research Corporation under Contract 68-03-3112 for ECAO, U.S. EPA, Cincinnati, OH.

APPENDIX B

Data Used for the Derivation of q_1^{*a}

Compound: Styrene

Species, Strain, Sex: mice, O_{20}, male and female

Body Weight: 0.03 kg (assumed)

Length of Exposure (l_e) = 112 days

Length of Experiment (L_e) = 840 days

Lifespan of Animal (L) = 840 days

Tumor Site and Type: lung/adenoma or carcinoma

Route, Vehicle: by gavage/olive oil

Experimental Doses or Exposures (mg/kg, once a week)	Transformed Dose (mg/kg/day)	Incidence No. Responding/No. Tested (or Examined)[b]	
		Males	Females
0	0	8/19	14/21
1350	25.7	20/23 (p=0.003)	-
1350	24.9[c]	-	31/32[c] (p=0.001)

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[a]Source: Ponomarkov and Tomatis, 1978

[b]Probability values for the Fisher's Exact Test are given after the incidence of tumors in dosed groups.

[c]Value is adjusted, as explained in text, to accommodate the multistage model.